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EDITED BY

GEORGE H. F. NUTTALL, F.R.S.

Quick Professor of Biology in the University of Cambridge

ASSISTED BY

EDWARD HINDLE, PH.D.

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Extract from Dr Rivers' Preface

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OBSERVATIONS ON *ENTAMOEBA HISTOLYTICA*.

BY D. WARD CUTLER, M.A. (CANTAB.),

Assistant Lecturer in Zoology, Victoria University, Manchester.

(With Plate VII and 1 Text-figure.)

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INTRODUCTION.

A VERY large number of publications dealing with *Entamoeba histolytica* have appeared during the past few years, but we still lack any detailed account of the various changes which this organism undergoes during its life history.

The elucidation of the meaning of these changes of form has been one of the objects of my research during the past two years. For many months I have been employed on the routine examination of the faeces of patients who were either suffering from amoebic dysentery or else were suspected of being carriers of *E. histolytica*. It was my practice during these examinations to make stained preparations of any amoebae found in the stools, and by this means I have been able to accumulate a large number of preparations for careful study.

Early in 1917 I devised a method (Cutler, 1918) by which the amoebae could be kept in artificial culture for many months. Six cultures were obtained and inoculated into cats, all of which died of typical amoebic dysentery.

These cultures and the material from the infected cats have enabled me to make a fairly complete study of the forms assumed by *E. histolytica*, and also

to perform various experiments which would have been difficult had I not cultural strains in my possession.

The "intra vitam" staining of the amoebae by a 1/10,000 solution of neutral red, described by Williamson and myself (1917), has proved a valuable aid in my researches.

METHODS.

The stained preparations were all made by the wet-film method. At different times I have tried most of the common fixatives, but obtained the best results with Schaudinn's sublimate alcohol mixture or with Bouin's fluid. For ordinary purposes the stains I employed were Heidenhain's iron haematoxylin and Dobell's modification described by him in 1914. This latter method is an extremely useful one, when rapid staining is desired, and the results are in every way equal to those obtained by the former. As counter stains to iron haematoxylin I have used alcoholic eosin, light green, and orange, G, all of which gave satisfactory results. I moreover employed at different times a number of other stains to those above mentioned, *i.e.* Ehrlich-Biondi, Delafield's haematoxylin and eosin, acid carmine and nigrosine, Fleming's tricolour, etc.

The study of the nuclear changes, which occur during the life cycle of the amoebae, was beset with difficulties, among them being our lack of precise knowledge as to the reactions of substances such as plastin and chromatin.

Methyl green acidulated with acetic acid was used with unfixed material, but Hertwig has cast doubt upon this stain being specifically chromatic; for he found that in *Actinosphaerium* the nuclear material was not affected, but the plastin framework was coloured.

I therefore tried the action of the various stains mentioned above. As a control in studying the staining effects I employed thin sections through the egg of the cockroach, which is known to possess a plastin nucleolus.

The following table gives the reactions to the various stains.

Stain	Plastin	Chromatin
Delafield's haematoxylin and eosin	Red	Blue
Ehrlich-Biondi	Maroon	Green
Acid carmine and nigrosine	Blue	Red
Fleming's tricolour	Yellow	Purple

I have realised, however, that staining reactions alone must not be too much relied upon and that as Minchin says (1912) "A given body or grain in the cell cannot be definitely identified as chromatin, in all cases, by any chemical or physical test, but only by its relation to the life and development of the organism as a whole, and more especially to the function of reproduction."

Keeping this standpoint in view I have not relied exclusively upon the staining effects of the various substances in the cytoplasm and nucleus of *E. histolytica*; but have based my conclusions primarily on the behaviour of these substances during the life cycle of the organism: using their staining

reactions only as supporting the conclusions so derived. The investigations which I have made upon the action of various substances upon the amoebae were carried out with a piece of apparatus devised by Cropper and Drew (1914):

"A plate of thin glass about three inches long and two inches wide has a narrow glass arm cemented to it along the short end, forming a projection upon which a small bottle can be hung by means of a piece of wire. The plate is covered over with a sheet of fine calico having a circular hole in the centre just large enough to permit of a cover glass resting on the plate with its edges almost touching the cloth all round. Two narrow strips of cigarette paper are placed on opposite sides of the aperture under the cover glass to prevent this from actually pressing on the slide....Water or the solution to be employed is placed in the bottle, and is slowly conveyed by capillarity along a piece of lamp wick to the cloth, which is in this way kept continually moist. The amoebae are put into a drop of the solution on the cover slip, which is then inverted and allowed to rest on the slide in the way described."

I have used this apparatus constantly, both for observing the free vegetative forms and also cysts, and have found it to be of great use.

LITERATURE.

The literature concerning *E. histolytica* is too vast to deal with here, moreover, most of the important papers have been summarised by others. It will suffice to outline the trend of previous research, reserving for the discussion at the end of the paper the points in which my results confirm or differ from those of other workers.

Until recently there were supposed to be three species of parasitic amoebae in the human intestine capable of causing dysentery: *E. histolytica* Schaudinn, 1903, *E. tetragena* Viereck, 1907, reinvestigated by Hartmann (1911), and *E. minuta* Elmassian, 1909. The accounts given of these organisms by the authors mentioned are well known and need not be dwelt upon. Darling, in Panama, demonstrated the identity of *E. histolytica* and *E. tetragena*. By inoculating cats he found that the large forms of the parasite, with the typical "histolytica"[1] nucleus, predominated at the beginning of infection, but later were replaced by forms with the "tetragena"[1] type of nucleus, which passed into the cyst condition. More recently it has been shown that the "tetragena" forms give rise to smaller amoebae, the precystic stage of *E. histolytica*, these being identical with *E. minuta* Elmassian. Thus the three so-called species of amoebae producing dysentery represent in reality but different stages in the life history of one organism.

Schaudinn also described a peculiar method of reproduction in *E. histolytica*, which he termed budding; he asserted, moreover, that small spores were

[1] While recognising that the original descriptions of the life cycle of *E. histolytica* were probably based on observations on degenerate amoebae, I propose to adopt the term "histolytica" type of nucleus to denote one poor in chromatin, such as was once thought to be characteristic of *E. histolytica*. On the other hand the term "tetragena" type of nucleus signifies one rich in chromatin and containing a large karysome typical of *E. tetragena*.

produced by the nucleus breaking up into chromidial granules, some of which passed into the spores, budded off from the peripheral cytoplasm. Darling and others have, however, shown that these budding phenomena must be regarded as degenerative.

During the past three years many papers have appeared, the majority dealing almost entirely with methods of treatment. Foremost among these papers are those by Wenyon and O'Connor in which many of the problems connected with *E. histolytica* and its pathological effects are fairly exhaustively treated. Dobell and Jepps, Fantham and Porter, Malins Smith and others record observations on the cysts of *E. histolytica*; and a long series of papers have appeared in French journals. Many of these latter, however, have tended to obscure the problem by drawing sweeping conclusions from very scanty material.

EFFECTS OF VARIOUS SUBSTANCES ON AMOEBAE IN CULTURE.

The interesting results obtained by Cropper and Drew (1914) by subjecting free-living amoebae to the action of various substances led me to make a few similar experiments with cultural *E. histolytica*.

The substances, whose action I wished to test, were made up to the required strength in the culture media; and the wet chamber apparatus, already described, was employed. In all cases control slides were used.

Chlorine. A 12 hours' subculture was used for making four preparations: (A) served as a control, (B–D) were placed in wet chambers with chlorine as follows, the number of amoebae on the slides was counted before and after incubation at 30° C.:

	Strength of Cl employed	No. of amoebae present at start	No. of amoebae present after 12 hrs incub.
A. Control	0	10	14
B. Chlorine	0·005 %	15	15
C. „	0·0005	12	21
D. „	0·0001	16	30

Five other experiments of a similar nature were made, and the results in all cases were strictly comparable. It thus appears that chlorine in the strength of 1/10,000 stimulates multiplication, whilst 1/2000 has less effect and 1/200 partially inhibits multiplication.

Wenyon and O'Connor found that chlorine 1/10,000 has no effect on *E. histolytica* cysts; it is of interest, therefore, that a similar solution stimulates propagation in the vegetative forms.

Tyrosin. Experiments, similar to the above, were made with strengths 0·2 %, 0·5 %, 0·01 %. The first two solutions stimulated multiplication, but the last as well as weaker solutions (0·005 %, 0·0005 %) had no appreciable effect. The following protocol relates to one, out of many experiments performed:

	No. of amoebae at start	No. of amoebae present after 12 hrs incub.
Control	15	18
Tyrosin 0·2 %	13	21
,, 0·5	18	24
,, 0·01	16	18

Skatol. This substance in the strength of 0·5 % rapidly induced cyst formation, practically every one of the amoebae becoming encysted: a few, however, died as a result of the experiment. These results were obtained in every one of the twelve experiments performed and in each experiment six control cultures containing no skatol were used. It is of interest in this connection to note that all the encysted amoebae possessed a nucleus typical of encysted amoebae from dysenteric stools (nucleus not of "histolytica" type). As amoebae with all types of nuclei occur in cultures, it appears possible that the forms killed by skatol had nuclei of the "histolytica" type. This seems likely because, in preparations stained with methyl green, the amoebae, which had not encysted, and appeared to be moribund, had nuclei very poor in chromatin and of "histolytica" type.

The significance of these observations will be more clearly seen by reference to page 141.

The influence of skatol in inducing cyst formation is of interest because this substance occurs naturally in the large intestine: as does also tyrosin, as a result of autolysis.

It is well known that in dysentery infections, at the base of the ulcers, there occur large tissue-invading forms of *E. histolytica*; while at the surface of the intestinal mucosa the "minuta" cyst-forming stage is frequently encountered. It is possible that the presence of skatol in the gut lumen may induce cyst formation, but it cannot be the sole cause, since in the cat's gut cyst formation probably never occurs, although skatol is present.

THE TROPHOZOITE.

APPEARANCE OF THE LIVING AMOEBA.

As previously stated, *E. minuta* represents the precystic stage of *E. histolytica*. This stage is dealt with under cyst formation (see p. 135).

The endoplasm of all stages is often granular in appearance, and stains a bright pink with a weak solution of neutral red. Forms, especially in cat infections, are very commonly found with a distinctly alveolated endoplasm, Plate VII, figs. 1, 2, the alveoli are irregular as regards size and are not evenly distributed. The endoplasm is distinctly marked off from the ectoplasm, and when neutral red is employed this demarcation is very apparent as the ectoplasm remains unstained. Numerous food particles are usually found in the endoplasm: the most common being red blood corpuscles, which are sometimes present in large numbers, fig. 3. Leucocytes and bacteria are also ingested.

PSEUDOPODIA.

The pseudopodia are blunt. When they begin to protrude, they are ectoplastic. Soon, however, the granular endoplasm flows into them; in this flowing movement there is a central stream of protoplasm, which bends round in all directions "fountain fashion," as soon as the periphery of the pseudopodium is reached. While this is taking place at the end opposite the pseudopodium, the endoplasm passes forward so that a slight progression of the whole animal occurs. At times, however, there is no translatory movement, although active pseudopodial formation is taking place. The text-figure below represents the movements of a culture form observed on the warm stage of the microscope. The pseudopodia were formed very rapidly, but from 3–30 to 3–35 there was no forward movement of the animal. This sudden development of pseudopodia from all parts of the body, unaccompanied

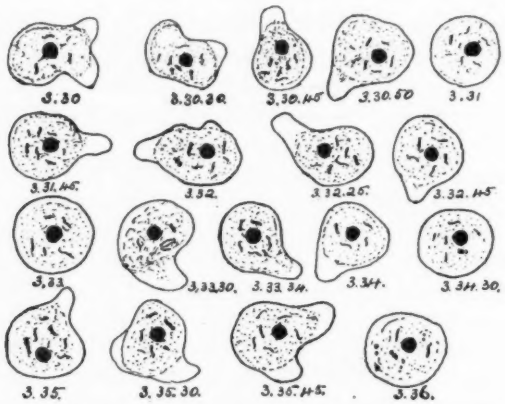

Fig. 1. Successive drawings made of the pseudopodial formation in a culture amoeba containing a blood corpuscle and many bacteria.

by progression, is a very characteristic feature of *E. histolytica*, as distinct from *E. coli*.

In stained preparations the pseudopodia are seen to be composed internally of a meshwork of protoplasm, which has apparently streamed out from the endoplasm as in the living organism, Plate VII, fig. 4.

FOOD INGESTION AND DIGESTION.

In the endoplasm of the amoebae a large number of blood corpuscles and other food particles are usually seen. Occasionally I have observed the method of ingestion of the blood corpuscles, which has always been that designated by Rhumbler (1910) as invagination. The amoeba on touching the object to be engulfed adheres to it, the part of the ectoplasm in contact with the food is invaginated into the endoplasm, forming a tube-like structure

whose walls ultimately fuse, leaving the food particle in the interior of the body. On reaching the endoplasm no change is observed for a period of time varying from 5 to 20 minutes, the longer period occurring when a relatively large body, such as a blood corpuscle, is undergoing digestion. Eventually, however, there is developed round the food a clear space, giving a very marked alkaline reaction to neutral red. Until this reaction sets in the food has apparently suffered no change except for the escape of haemoglobin, but soon after the onset of alkalinity the size of the food body diminishes until only a small portion remains, which is probably extruded from the cytoplasm.

Many observers have studied the process of digestion in Protozoa: Nirenstein (1905) found in Infusoria that there were two periods, firstly an acid one followed in a short time by an alkaline one. In *Actinosphaerium*, however, Greenwood (1894) found the acid period was omitted.

In *E. histolytica* the endoplasm normally has an acid reaction, as shown by the effect of neutral red. It is possible, therefore, that the part of digestion, performed during the acid period in the Infusoria, occurs in *E. histolytica* during the interval that elapses before the onset of alkalinity. It may well be that in *Actinosphaerium* also the endoplasm is normally of an acid nature.

MORPHOLOGY AND NUCLEAR DIVISION.

The average size of *E. histolytica* is usually given as 25–30μ, though Hartmann considers that 15-20μ is the most common size. Dimensions, however, are of no value for the determination of species, as there is a wide range. For instance I have found forms measuring only 13μ which were undoubtedly *E. histolytica*, together with other amoebae which were 30–35μ in diameter. These measurements are certainly extremes, but size variability is as characteristic of *E. histolytica* as of other Protozoa.

The resting nucleus is usually round or oval in shape and invisible in the living condition. Stained preparations show a fairly thin membrane on which chromatin is situated in the form of a thin layer. In the centre there is a karyosome, from which fine threads radiate to the periphery, and on these threads chromatin granules are usually found, Plate VII, fig. 5.

The karyosome appears as an evenly stained matrix, but occasionally chromatin granules are found distributed through it.

From the staining reactions and also from the subsequent history I conclude that this central body is composed of both plastin and chromatin.

This form of nucleus is typical for *E. histolytica*, when it is in the trophozoic condition.

The changes which precede division are characteristic. The karyosome loses its compact form, becoming differentiated into chromatin granules and an homogeneous mass, composed almost entirely of plastin, Plate VII, fig. 6. Often the central plastin body is surrounded by a clear zone bounded by granules from which threads pass to the periphery, Plate VII, figs. 1, 9. The disposition of the granules, however, is quite indiscriminate, sometimes they

form a compact mass on the plastin, Plate VII, fig. 6, while at other times they are irregularly distributed through the nuclear sap.

During these changes the marginal chromatic band disintegrates into granules, which give a chromatin reaction to methyl green and other nuclear stains.

Later there is extrusion of chromatin grains into the cytoplasm. Plate VII, Fig. 10, shows this process; the central mass of the nucleus has aggregated to the side from which extrusion is taking place. This is not always the case, however, for often the karyosome remains centrally situated. The study of many preparations showing this phenomenon has convinced me that the extruded chromatin is derived from that found on the nuclear membrane.

On reaching the cytoplasm the granules usually aggregate into an irregular number of small bodies, figs. 7, 11, 12, 14, which often persist until the nucleus has divided, fig. 22. Their chemical nature seems, however, to undergo a change, for when extrusion occurs, as in fig. 10, they stain a distinct purple with Fleming's tricolour stain and green with methyl green; but on reaching the cytoplasm, as in figs. 7, 11, 12, 13, 14, they stain red with the tricolour stain and remain unaffected by methyl green.

After this process the nucleus becomes smaller and appears as a lightly stained body with a few peripheral granules. In the centre there is a distinct plastin body, with granules, figs. 12, 13, or the granules lie between the plastin body and the nuclear membrane, figs. 7, 14. On occasion two of these grains can be seen side by side as in fig. 15, and could be interpreted as the two portions of a divided centriole. I believe that figures similar to this led Hartmann to the conclusion that centrioles are found in the dividing nucleus of *E. histolytica*. I may state at once that I have found no justification for such a view.

Finally another variation occurs where in the centre of the nucleus there is the plastin body with a few granules and the remainder on the periphery, Plate VII, figs. 2, 11.

Division stages are extremely rare and I had to examine very many individuals before I was able to work out the different phases. I endeavoured to watch the process in the living animal but with no success. On two occasions I was able to see the division of the animal, but as the nucleus was not visible during the process it was impossible to note the details. The following description is therefore based entirely on stained preparations. At the beginning the nucleus elongates and some of the peripheral chromatin becomes massed together at each pole. The plastin mass in the centre also elongates and the chromatin grains aggregate together on and around this body, Plate VII, figs. 4, 16. Very fine spindle fibres are formed radiating from one end of the nucleus to the other, Plate VII, fig. 17. I have not been able, however, to discover from what part of the nucleus these fibres are produced. The chromatin granules arrange themselves on the spindle fibres and finally approximately equal numbers pass to each pole of the nucleus; at the same time the plastin body

becomes constricted in the middle, Plate VII, fig. 18, and ultimately divides. The result of these processes is an elongate nucleus possessing at each pole a granular mass of chromatin together with some plastin substance, Plate VII, figs. 19, 20.

Finally the nuclear membrane constricts in the middle, and ultimately divides into two halves, so that two small nuclei are formed, closely resembling that of the parent, Plate VII, figs. 21, 22.

The nuclei now separate from each other.

The division of the cytoplasm does not occur directly after that of the nucleus, for two-nucleate amoebae are commonly found and the divided nuclei undergo reconstruction, by a reversal of the process mentioned above, so that the resting type is produced before the animal divides into two.

When this takes place the cytoplasm constricts so that two approximately equal animals, each containing a nucleus, are formed. I have never observed the division of an amoeba into more than two, and have no evidence for the belief that there is reproduction by schizogony, where a four-nucleate amoeba breaks into four small merozoites.

The organisms produced by binary fission have two courses open to them, they may increase in size and become tissue invading forms, or the growth may be small, in which case they constitute the cyst-forming amoebae described by Elmassian as *E. minuta*.

Of what determines which of these courses shall be pursued I have no definite knowledge.

CYST FORMATION.

The amoeba preparing to encyst is much smaller than the tissue-invading form just described. Elmassian gives the size of these small animals as 12–14 μ, but this figure is a little high, for after measuring a large number of individuals I find a range from 9–12·5 μ, with an average size of 12·1 μ.

Dobell and Jepps (1918) with other workers have described a small variety of cyst measuring 10 μ or less, which is identical in structure with the normal type. It is suggested that these small cysts are produced by a small strain of *E. histolytica*[1].

During the course of my work cysts of this small type were fed to a cat, which developed dysentery and died. Preparations of the intestine of this cat showed the presence of a small variety of tissue-invading amoebae, but none of the normal forms have as yet been seen. I have not, however, examined sufficient material to be able to state that no large *E. histolytica* are present in the tissues.

The general morphology of the cyst-forming amoeba is similar to that of the tissue-invading type; the endoplasm may be alveolated or granular and filled with food particles, consisting of blood corpuscles and bacteria, but I have never

[1] While this paper was passing through the press Dobell and Jepps published a detailed account of size variations in cysts (*Parasitology*, vol. x, 320). The same subject has been dealt with by Malins Smith (1918) in the *Annals of Tropical Medicine and Parasitology*, XII.

seen any ingested leucocytes. When at rest it is difficult to see a well-defined ectoplasm, but this becomes very apparent when pseudopodia are formed, which are like those of the large amoebae.

The nucleus is not visible in the living condition, but after staining it is seen as a small round or oval body with an average size of 4μ. In structure the nucleus is identical with that of the large non-dividing forms, Plate I, figs. 23, 24.

Before encystment food particles disappear from the cytoplasm, and the animal becomes spherical or slightly oval in shape. Round the whole of the body a cyst wall is formed.

Nuclear changes occur similar to those described above; the whole mass increases, but the karyosome becomes a little smaller and many granules are seen between it and the nuclear membrane, Plate VII, fig. 25. Often these arrange themselves in one or two semilunar masses on the membrane, Plate VII, fig. 26. A process of chromatin extrusion now takes place as in the tissue-invading forms, Plate VII, fig. 27.

After these changes the nucleus becomes a little smaller, much of the peripheral chromatin disappears and the karyosome is seen as a small homogeneous mass, on which granules are situated, Plate VII, fig. 28. During these processes a large vacuole developes in the cytoplasm of the cyst, Plate VII, fig. 26; on occasion one or two vacuoles have been seen, Plate VII, figs. 29, 30, and the characteristic structures known as chromidial blocks or chromatoid bodies make their appearance.

The division of the nucleus is identical with that of the vegetative forms. The various stages are seen in Plate VII, figs. 29, 31, 32, 33. Fig. 34 shows the end of the process where the two daughter nuclei are connected by a thin chromatin thread.

I have occasionally seen a cyst in which the two nuclei were of unequal size. This condition is probably brought about by the final stage of division being unequal, but I have never observed the process leading to this condition.

After the separation of the nuclei they are characterised by the possession of a small central karyosome, composed of plastin and chromatin granules, and a thin nuclear membrane on which other granules are situated. The space between the membrane and the karyosome is also occupied by these bodies, Plate VII, fig. 35. Binucleate cysts are not common, probably because the two nuclei, soon after their formation, divide once more to form the characteristic quadrinucleate cyst of *E. histolytica*.

This division is like that already described, but is not preceded by nuclear reconstruction. Plate VII, fig. 36, shows the final stage where four daughter nuclei are seen connected together in pairs by a narrow strand of chromatin: each containing a central body and granules distributed through the nuclear sap. After the nuclei become free in the cyst they reconstruct themselves by some of the granules becoming incorporated with the central body and the remainder passing to the nuclear membrane. Thus a quadrinucleate cyst

similar to that seen in Plate VII, figs. 37, 38, is produced. The manner in which the nuclei lie in regard to one another is quite indiscriminate. As this nuclear reconstruction is proceeding the vacuole, with its contained glycogen, gradually disappears, but the chromatoid bodies may or may not be present.

CHROMATOID BODIES.

These structures were originally supposed to have their origin in the chromatin extruded from the nucleus; this was definitely stated to be the case by Hartmann in his paper on *E. tetragena* and also by Elmassian in *E. minuta*.

Recently, however, there has been great doubt as to the accuracy of this statement: thus in a recent publication Dobell and Jepps (1917) make the following remark: "these bodies, which we shall call chromatoid bodies (since in our opinion they have nothing to do with the chromidium of other rhizopods, and are not crystalloid) are, it is true, very generally—though by no means invariably—present in *E. histolytica* cysts."

With this view I am in complete agreement. As regards the origin of these bodies I am quite ignorant, although a good deal of time was spent in trying to discover it. I am convinced, however, that they are not produced from the extruded chromatin, for in many cases the chromatoid bodies can be seen in the cytoplasm of the cyst before the nucleus has completed its extrusion of chromatin, Plate VII, fig. 27: also it is possible to see, at late stages of development, these bodies and the extruded chromatin lying side by side in the cyst, Plate VII, figs. 33, 35. Finally the volume of the chromatoid bodies is so great as to preclude the idea that the entire mass of their substance could have been derived from this cast-out chromatin.

When stained with iron haematoxylin they appear like chromatin, but methyl green does not affect them: moreover with Fleming's tricolour they appear as red bodies in contrast to the purple coloured nuclear chromatin. With the recognised crystalloid stains the reaction is not characteristic of crystalloid substances.

FURTHER DEVELOPMENT OF THE CYST.

What occurs when the ripe cyst enters another host has been the subject of much speculation by protozoologists. In *Entamoeba blattae* Mercier (1910) has shown that small amoebulae are set free from the cyst, which conjugate, and give rise to the vegetative forms. That a similar process takes place in other parasitic amoebae has been suggested many times.

I have not been able to demonstrate with certainty that *E. histolytica* behaves in this way, but many of my results indicate that some such process does occur. Further experiments are being carried out, but it seems worth while to mention here the probable sequence of events, pending the publication of my more complete results.

Penfold, Woodcock and Drew in 1916 announced that they had devised a method by which *E. histolytica* could be artificially induced to encyst. This was accomplished by the use of a solution of "Liquor pancreaticus" (Benger). From the cysts thus treated only one amoeba emerged, but whether it was uninucleate, binucleate, or quadrinucleate was not stated.

These experiments I repeated with similar results except that the proportion of cysts which reacted to the treatment was small; further all the excysted amoebae were uninucleate. Further experiments have convinced me however that my results were pathological and due to the ferments dissolving the thin cyst walls of amoebae which had just previously encysted. Dobell at this time also suggested to me that this was the explanation of the somewhat extraordinary result. A slight modification of the method adopted by Woodcock, Penfold and Drew has, however, proved more satisfactory. If a solution of "liquor pepticus" is first allowed to act on the cysts for a short time, followed by a similar solution of "liquor pancreaticus," a very large proportion of the treated cysts react. Of the excysted amoebae some are uninucleate, as in the previous experiments, but the majority are quadrinucleate, Plate VII, fig. 39. These forms I consider represent those which would emerge from the cyst under normal conditions, they move actively on the warm stage of the microscope, but though watched for five or six hours, I have not been able to observe them take any food or to determine definitely what is the next phase of their life cycle.

That they are produced from *E. histolytica* cysts is evidenced by their general appearance, and by the fact that I have been able to stain them with methyl green during their emergence.

The cysts, which did not react to treatment, were in the vast majority of cases binucleate.

Although I have not seen the intermediate stages there is evidence that these quadrinucleate amoebae ultimately divide to form four small amoebulae, such as shown in Plate VII, fig. 40.

Whether these amoebulae are gametes and conjugate, as in *E. blattae*, I cannot say at present.

DEGENERATION IN *E. HISTOLYTICA*.

As stated on p. 129, Schaudinn described a method of sporulation in *E. histolytica*, where the nucleus was converted into chromidia. Some of these chromidial granules passed to the periphery of the cytoplasm, which aggregated round them to form small buds, 3–7 μ in size. These buds became detached, secreted round themselves a thick wall, and became the spores or cysts of the parasite.

Protozoa living in artificial culture are liable to undergo degeneration and *E. histolytica* has proved to be no exception to this rule: I have therefore made a full study of degeneration in culture forms and compared them with similar amoebae in the faeces of infected men.

As others have noted in the first stage in degeneracy the nucleus increases in size and the chromatin aggregates into clumps, Plate VII, figs. 41, 42. In some cases these masses are confined to the periphery, but more commonly they are found scattered throughout the nucleus. The animal does not increase very markedly in size, but large vacuoles often fill the granular cytoplasm.

When these degenerate organisms are studied in the living condition the nucleus is easily seen, the masses of chromatin appearing as refractile bodies; this suggests that the chromatin has changed its chemical nature, as was noted by Dobell (1909) in degenerate forms of *E. ranarum*. In *E. histolytica* the amoebae are quite active, but they appear incapable of ingesting food substances.

Nuclear division, of an irregular type, often occurs, so that the animal arrives at a condition seen in Plate VII, figs. 43, 44, with six or more degenerate nuclei.

Elmassian in his paper on *E. minuta* has figured an amoeba, fig. 20, with dividing nuclei, which strongly recall the divisions seen in fig. 43. This observer did not, however, regard them as degenerate.

The next stage in degeneracy is the disappearance of the nuclear membrane and the passage of the chromatin masses into the cytoplasm, Plate VII, figs. 45, 46, 53. The production of brown pigment, described in *E. ranarum*, I have not seen.

Finally enucleate animals are produced, the cytoplasm containing highly refractile granules with staining reactions similar to those of chromatin, Plate VII, figs. 47, 48. These organisms may live for hours, moving actively the whole time.

Small buds, containing refractile grains, are very commonly produced, Plate VII, figs. 49, 50, and correspond with the spores described by Schaudinn.

In rare cases the nucleus, instead of immediately resolving into chromatin masses, becomes uniformly stained. Plate VII, fig. 51, shows two such nuclei lying together in a vacuole into which chromatin is being extruded.

Appearances such as these doubtless led Hartmann to postulate the existence of autogamy, where there is fertilization of two reduced nuclei. This supposition he has since abandoned.

Finally the two nuclei undergo further disintegration until a condition is reached seen in Plate VII, fig. 52. The end result is for the nuclei to disappear completely leaving a few chromatin granules in the cytoplasm.

Temperature has a decided effect in bringing about degeneration, for if a culture, in which the number of these forms is few, be incubated at 22° C. instead of at 32–37° C., the great majority of the amoebae pass into this condition in a few hours.

Thermal change cannot, however, be the only operative factor, for cultures incubated at the same temperature (32° C.) vary greatly; in some the degenerate individuals are common, while in others no such forms, or at any rate very few, are found.

Similarly it can be shown that excess or deficiency of food supply does not produce degeneration, for cultures apparently identical, as regards available food, will vary considerably. It must be recognised, however, that cultures containing many forms of bacteria, as my cultures do, are not living under the same conditions; and the various bacterial products may be very different both quantitatively and qualitatively in the various cultures.

Probably these substances have a decided influence in causing degeneration.

Dobell, from studies on *E. ranarum*, concluded that temperature, together with an unknown factor preventing the extrusion of chromidia, is the cause of degeneration.

This suggestion seems applicable to *E. histolytica*, for the incubation at a lower temperature than the normal would cause nuclear overgrowth, which for some reason or other (bacterial products might here come into play) is unable to be compensated by the extrusion of the excess chromatin into the cytoplasm. Possibly the irregular nuclear divisions are an expression of this need on the part of the organism.

Further it is significant that in cat infections, where cyst formation with chromatin extrusion is rare, degenerate amoebae are common. Such amoebae often fuse together. Plate VII, fig. 54 was drawn from a preparation made from the faeces of a patient, while fig. 55 represents fused amoebae in the lumen of a large intestinal tubule of an infected cat. The nuclei exhibit degeneration changes.

Degeneration recorded by other Observers.

Hartmann dealing with *E. tetragena* distinguished two types of degeneration forms. In the first the nucleus was swollen with large clumps of chromatin, while in the second the nuclear membrane disappeared. I think from my work that it is evident that this second type is the continuation of the process begun in the first type. Darling considers that the buds so often seen in *E. histolytica* are due to degeneration, a view in which I thoroughly concur.

Bartlett in a recent communication has described cells, often associated with *E. histolytica*, which he termed refractile cells. His figures suggest that they are degenerate amoebae.

The active movement, which these bodies exhibit, is no bar to this view.

The following is the description which Bartlett gives of the refractile cells "In unstained films they are extremely well-defined rounded cells, which are more refractile than the body cells, but less refractile than the vegetative entamoebae; they are greyish rather than greenish in colour. They vary in size from that of a neutrophil leucocyte to a large phagocytic endothelial cell. The nuclei may be single and of small size, or may be multiple. When multiple they may be two, four or more in number, and may be equal or unequal in size. The nuclei show characteristic crescentic thickenings on their margins."

As will have been noted this description, especially as regards the nucleus,

very closely resembles that which I have given for the degenerate forms of *E. histolytica*.

Dobell's account of the process of degeneration in *E. ranarum* is essentially similar to that which I have described.

Finally Prantl has noted similar degenerative changes in *Amoeba proteus*, which he attributes to active multiplication caused by a prolongation of suitable conditions, which normally would have disappeared.

DISCUSSION.

Firstly let us consider the meaning of the various form changes, especially of the nucleus, which are to be found when many *E. histolytica* amoebae are examined.

The above observations show that the large amoebae with nuclei of the "tetragena" type are trophozoic forms incapable as yet of undergoing division. For this to take place the nuclear changes occur, so that a nucleus is produced poor in chromatin and with a small karyosome. This type is similar to that described for *E. histolytica* by the earlier workers. It is indubitable that the majority of the phases described for *E. histolytica* were degenerative ones, but I think it highly probable that the nucleus described as typical for *E. histolytica* was not degenerate, but ready for division.

Thus it appears that the nuclei described as characteristic of *E. histolytica* and *E. tetragena* must be considered as phases in the life cycle of the same organism.

Darling observed that at the beginning of infection in cats most of the amoebae had a nucleus of the "histolytica" type, and "tetragena"-like nuclei were produced as the infection continued. Also cats fed with cysts developed dysentery after a short incubation period and the amoebae, though first "tetragena"-like, soon passed into the "histolytica" condition. This is exactly what one would expect, if the "histolytica" type of nucleus is the one preparatory to division, as I believe it to be; for at the beginning of infection active multiplication would occur giving rise, as infection continued, to "tetragena"-like resting forms.

Dale and Dobell (1917) state that during 43 passages through kittens of a strain of *E. histolytica*, they observed no metamorphosis of the amoebae as Darling described, where the parasite passed from the "histolytica" to the "tetragena" and finally to the "minuta" form at which stage the infectivity by rectal inoculation ceased. Further these workers agree with Wenyon, Baetjer and Sellards "that a strain of amoebae in the cat remains constant and can be propagated indefinitely."

If Dale and Dobell mean by this statement that they found no evidence of cysts formation in cats, I am in entire agreement. I cannot believe that they deny the change from the "histolytica" to the "tetragena" type of nucleus, for it is so evident in cat infections and also in amoebae from the human intestines.

I find no evidence for the statement of Swellengrebel and Schiess that the

nucleus of *E. histolytica* in the cat is different in structure from that found in amoebae from the human intestines.

Turning now to consider the details of nuclear division the account given is, I think, the first in which the change undergone by the karyosome is worked out with any degree of completeness. The division is a primitive one and broadly similar to that described by others, but the behaviour of the constituent parts of the karyosome, namely the plastin and chromatin, is peculiar and of interest.

The extrusion of chromatin into the cytoplasm of the vegetative forms is also of interest.

There is no trace of centrioles in the dividing nucleus, nor of the extra-nuclear centriole described by Elmassian: I conclude therefore that the announcement of their existence was due to errors of observation.

Figures similar to those given by Elmassian of extra nuclear centrioles are commonly found, but in all cases the granules, superficially resembling centrioles, are found to be either small cocci, which have been ingested, or other cytoplasmic inclusions in no way connected with the nucleus.

Nuclear changes have been described by Hartmann under the term nuclear cycle, but the significance and relation, which they bear to division, do not appear to have been clearly recognised by this observer. In his paper on *E. histolytica* he states that this cycle of the nucleus rarely occurs, a fact readily understood when it is remembered that the "histolytica" type of nucleus is one ready to divide. As regards the main outlines of the life history of the vegetative forms I am in agreement with the majority of workers, except that I have been unable to find evidence for reproduction other than binary fission in the vegetative amoebae.

The general morphology of *E. histolytica* is so similar to that of *E. ranarum* that Dobell in 1914 suggested that the life history of the two parasites was probably almost identical. Further the differences between the two seemed to him so small that he considered it possible that they were one and the same species. To test this idea experiments were performed, which have been published in a recent number of *Parasitology* (1918). The result, however, was negative and Dobell concludes "that *E. histolytica* Schaudinn and *E. ranarum* Grassi are probably distinct species, and consequently the frog in all probability is not a reservoir of human amoebic dysentery." I would, however, point out that there are a few morphological differences between the two forms, which render this view of their non-identity probable.

The cytoplasm is similar, although in *E. ranarum* the ectoplasm and endoplasm are not distinctly marked off from each other. The nuclei of the vegetative forms are, however, dissimilar, thus in *E. ranarum* the nucleus is devoid of a karyosome.

In the cysts extrusion of chromatin from the nucleus occurs and a karyosome developes by the running together of granules. This karyosome is obviously different from that of *E. histolytica*.

Division in *E. ranarum* is initiated by outgrowth at the poles, which draw apart, the karyosome lying at first in the middle of the spindle. Fibres are then developed and chromatin granules dispose themselves upon the threads, and pass to the opposite poles.

The daughter karyosomes are reformed by the granules rearranging themselves.

Obviously this method of division, though agreeing in the main, differs in detail from that found in *E. histolytica*.

Finally the division of the two nuclei into four in *E. ranarum* is different from the same process in *E. histolytica*, for in the latter the method is identical with that pursued by the original nucleus when dividing to form a binucleate cyst; while in the former there is a difference in the method of division to form the four-nucleate cyst.

These differences, though perhaps small, are sufficient to support the conclusion that on morphological grounds *E. histolytica* and *E. ranarum* are two distinct species.

Since Dobell published his account of *E. ranarum* a paper has appeared by Collin, in which the nuclear divisions of the vegetative forms, not recorded by Dobell, are described.

Collin's account is as follows: "étirement du noyau en un fuseau *à pôles aigus*, formation de fibres fusoriales aux dépens du karyosome, etc. Dans la très grande majorité de cas, l'aspect était celui d'une amitose complète: le noyau devenant légèrement elliptique, puis en biscuit, puis en haltère, le karyosome (cause mécanique sans doute de toute la division) s'étire de la même façon, sans perdre à aucun moment son individualité, ni même sa structure granuleuse, sinon en sa région moyenne qui devient fibrillaire comme dans le cas classique d'Amoeba crystalligera Gruber."

It will be seen that this description is very like that given for *E. histolytica*, especially as regards the karyosome, though Collin has not apparently differentiated clearly between the chromatin and plastin. The retention of individuality throughout the whole process is, however, one of the striking features of the karyosome in the nuclear division of *E. histolytica*.

Collin's further account, however, of the production of multinucleate schizonts in the larvae of Batrachians finds no parallel whatsoever, in any phase of the life history of *E. histolytica*.

In conclusion I wish to express my thanks to Prof. Dean for performing the inoculation experiments for me, and especially to Prof. S. J. Hickson who has read the manuscript of this paper and offered many valuable suggestions.

SUMMARY.

1. Observations on *E. histolytica* from the human intestine have been made and compared with amoebae living in culture media and in the ulcers of the large intestines of cats, which have died of amoebic dysentery.

2. The effects of the substances chlorine, tyrosin, and skatol on culture amoebae have been investigated; it has been found that the two first substances stimulate vegetative reproduction, but that skatol induces cyst formation.

3. The changes that the nucleus undergoes preparatory to division and the behaviour of the karyosome during division are fully described. It is shown that the nuclei originally described as characteristic of *E. histolytica* and *E. tetragena* are phases of nuclear change in the life history of the same animal.

4. Cyst formation and the nuclear divisions in the cysts are described, together with a short discussion as to the view taken that the chromatoid bodies are not comparable with the chromidia of other Rhizopoda.

5. Reasons are adduced for the suggestion that during the further development of the cysts quadrinucleate amoebae emerge, which divide to form four small amoebulae.

6. Degeneration in *E. histolytica* is described and the view of Darling, that budding phenomena are degenerative ones, is confirmed.

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DESCRIPTION OF PLATE VII.

The figures have been drawn from permanent preparations with the aid of a Camera Lucida and the following optical apparatus: Zeiss apochromatic oil immersion objective 2 mm. (N.A. = 1·3) and compensating oculars 6, 12 and 18. Critical illumination was always employed.

Figures 1–27, with the exception of figs. 2, 14, 15, 16, 17, are drawn from specimens fixed with Schaudinn's sublimate alcohol, the others are from specimens fixed with Bouin's fluid. Figs. 7, 11, 14, 22 are from specimens stained with Fleming's tricolour, the remainder from specimens stained by Heidenhain's iron haematoxylin method. The magnification of figs. 1–22 approximately 850 diameters, figs. 23–27 approximately 1800 diameters.

Figs. 1–3. Vegetative amoebae showing different appearances of the karyosome, and the alveolated cytoplasm.

Fig. 4. Amoeba with pseudopodia composed of a meshwork of endoplasm.

Fig. 5. Vegetative form with typical "tetragena" nucleus.

Figs. 6–14. Successive stages preparatory to division, all with the exception of fig. 14 from the human intestine, fig. 14 from a culture form. In fig. 11 the "histolytica" type of nucleus is seen.

Fig. 15. Specimen from cat; karyosome with two chromatin granules, which might be interpreted as a divided centriole.

Figs. 16–20. Successive stages in division of the nucleus, specimens from human intestine. In fig. 20 the nucleus is abnormally large.

Fig. 21. Two nucleate amoebae from a culture form.

Fig. 22. Two nucleate amoebae from the human intestine, extruded chromatin in the cytoplasm.

Figs. 23, 24. Precystic stage, *E. minuta*, fig. 23 from specimen in faeces: the nucleus is abnormally large. Fig. 24 from culture form.

Figs. 25, 26. Nucleus preparing to divide in the cyst, in fig. 26 the chromatoid bodies and vacuole have appeared.

Fig. 27. Nuclear extrusion.

Figures 28–38 were drawn from specimens fixed with Schaudinn's sublimate fluid and stained with Heidenhain's iron haematoxylin. Figs. 28–38 are magnified approximately 1800 diameters: fig. 40 approximately 2500 diameters and fig. 39 approximately 1000 diameters; the remainder approximately 850 diameters.

Figs. 28, 29, 31–34. Stages in division of the nucleus.

Fig. 35. Binucleate cyst.

Fig. 36. Last stage in division of the two nuclei to form the quadrinucleate cyst.

Figs. 30, 37, 38. Quadrinucleate cysts.

The above figures have been drawn from specimens from the human intestine.

Fig. 39. Quadrinucleate amoeba, which has excysted.

Fig. 40. Small uninucleate amoebula, probably derived from a quadrinucleate amoeba seen in fig. 39.

Figs. 41–53. Degenerate vegetative amoebae.

Figs. 41, 42. First stage of degeneration.

Figs. 43, 44. Irregular divisions of the nuclei to form multinucleate organisms.

Figs. 45, 46. Disintegration of the nuclear membrane.

Figs. 47, 48. Amoebae in which nucleus has broken up into refractile granules.

Figs. 49, 50. Bud formation.

Fig. 51, 52. Degenerate nuclei lying side by side undergoing degeneration and disintegration.

Fig. 53. Degenerate amoeba with disintegration nucleus.

Fig. 54. Association of degenerate amoebae; specimen from the human intestine.

Fig. 55. Similar association of degenerate amoebae and leucocytes found in a tubule of the large intestine of the cat.

Figs. 41, 43, 46, 47, 49, 53 are drawn from culture amoebae; figs. 42, 44, 45, 48, 50, 51 and 52 are drawn from specimens in the human intestine.

A REVISION OF THE COCCIDIA PARASITIC IN MAN.

By CLIFFORD DOBELL, F.R.S.

(*Imperial College of Science.*)

(With Plate VIII and 2 Text-figures.)

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INTRODUCTION.

The following memoir has grown gradually from very small beginnings until it has now assumed almost monographic proportions. Its history is as follows. Last year I accidentally discovered the oocyst of a coccidium in human faeces, and my attempts to identify it led me to study afresh the works of others dealing with the intestinal coccidia of man. From this I was led, little by little, to inquire into all the recorded cases of human coccidiosis which I could find. I had already studied this subject in some detail in the course of earlier work, and had perceived that our knowledge of the coccidial parasites of man was in a highly unsatisfactory condition; but I hardly realized, and I think few others even now fully realize, the state of decay into which it had fallen. Until the appearance of Wenyon's papers in 1915 hardly any facts had been definitely established concerning the coccidia of man; and the interpretations put upon such observations as had been recorded were—as can now, I think, be shown—for the most part wrong. But the new facts discovered by Wenyon have now made it possible to revise and correct our knowledge of the whole group of human parasites, and to place the organisms themselves on a secure

systematic foundation. The pages which follow embody my attempts in this direction.

I have recently been able to read, or re-read, almost all the works dealing with the coccidia of man. This has been no small task, for much of the literature is difficult of access; and I infer, from what I have read, that most people who have previously handled this subject have been deterred by this difficulty from consulting many of the original works. It is therefore almost superfluous to add that much of our "knowledge" has hitherto consisted of garbled versions of original records eked out with a number of groundless text-book traditions. No excuse seems necessary, therefore, for publishing this attempt to sift the facts and ascertain the truth.

Through the kindness of my friend Dr C. M. Wenyon (now Lieut.-Col., R.A.M.C.), I was able to see the coccidia which he described from man in 1915. Since then I have seen and studied one of his forms anew, and have found another and apparently unrecorded species which will be described later in the present memoir. As there has hitherto been much doubt regarding the relation of the human coccidia to those occurring in certain animals, I have also re-examined the species in question in order to supplement my knowledge of these organisms and to corroborate and check the opinions which I had reached, concerning the parasites of man, from my study of the literature and of the parasites themselves.

My researches on the coccidia—begun in 1906, and continued intermittently ever since—have given me a fairly extensive acquaintance with the group as a whole, and with the works, both ancient and modern, dealing with them. Without this knowledge I could not have attempted the present revision, nor should I have been able to express an opinion, on certain matters, with a degree of confidence such as the reader may sometimes observe in the following pages. I have dealt with much of the previously recorded work on the coccidia of man in a very critical spirit, because I wish to show how little is really and rightly known about this interesting group of forms, and how large a field there is, in consequence, for the activities of future investigators. If the present work succeeds in unravelling some of the old tangles, and provides a clue for other workers, who have the opportunity, to follow up, it will have served its purpose. None can be more conscious than I am myself of its numerous shortcomings.

To obtain the original works describing the coccidia of man, and those related to them, I have had to ransack many of the large libraries in London and elsewhere, and I take this opportunity of recording my indebtedness to the librarians who have everywhere aided me most willingly. It would be tedious to enumerate all the libraries[1] which I have made use of, and to thank

[1] For the benefit of others I should like to mention, however, that the works of Rivolta—which are of considerable systematic importance—have proved most troublesome to obtain; but I finally succeeded in discovering copies of them in the library of the Royal College of Veterinary Surgeons, and should like to express my thanks once more to the Secretary, Mr F. Bullock, who recently unearthed them and enabled me to study them there.

again individually all the friends who have, at one time and another, helped me to obtain works which are not readily accessible. I would ask them collectively, therefore, to accept again this expression of my gratitude. I am further beholden to several of my friends for information or assistance, which will be acknowledged in the proper place. I must, however, make special mention here of my indebtedness to Captain F. W. O'Connor, R.A.M.C., who—when he found that I was engaged upon the present work—most magnanimously placed at my disposal all his unpublished observations upon the *Isospora* of man. They were naturally of great interest to me, and I have availed myself of his generosity to the extent of incorporating some of his results, with due acknowledgments, in my account of *I. hominis* (Part II, sect. 1). I gladly offer him my thanks again here for his unselfish action.

I have only to add that I have completed the present work, and made most of the original observations recorded in it, whilst working with the aid of grants from the Medical Research Committee. Without this assistance, and the experience and knowledge gained whilst working on their behalf, the work itself could certainly never have been undertaken.

PART I.

HISTORIC AND ANALYTIC.

The history of our knowledge of the coccidia of man is difficult to write. It is complicated by the circumstance that our knowledge of the Coccidia, as a group, is comparatively recent[1], whilst most of the original cases of human coccidiosis were recorded during the early period of ignorance and uncertainty, and usually by workers with very scanty knowledge of the organisms related to those which they described. The older accounts are thus frequently written in an archaic language which is not easily comprehended—or which is, at least, apt to be misunderstood—by a modern worker unversed in the history of this particular branch of protozoology. To add to the difficulties of exposition, the coccidia of man are related to, and have frequently been confounded with, those of other animals; and these latter coccidia are unfortunately among the species which have changed their names periodically. We have thus to cope not only with discrepancies and difficulties in matters of fact, as recorded by different observers, but also with a confusing change and interchange of names which has thrown the nomenclature of the entire group of species into a state of chaos.

In considering the cases and findings already recorded, I have thought it best to divide my analysis into two distinct parts—one dealing with the work done prior to the year 1915, and comprising therefore all the older observations; the second dealing with the observations recorded from 1915 to the present day. I adopt this procedure because the year 1915, in which the observations

[1] The life-history of the coccidia, it may be recalled, was not properly understood until the publication of the celebrated researches of Schaudinn and Siedlecki in 1897.

of Wenyon were published, marks a turning point in our knowledge of the coccidia of man. After this preliminary analytic section, I will attempt to deal with the resultant facts in a synthetic manner, and to show as clearly as possible the present state of our knowledge of the whole subject.

A. The Human Cases of Coccidiosis described prior to the year 1915.

In dealing with the records of coccidial infection of human beings, I shall first consider the observations of each worker separately, and as far as possible in historic sequence. I may remind the reader, however, before doing so, that the name *Coccidium*—now, as a generic name, generally replaced by *Eimeria*—was introduced by Leuckart in 1879, and that previous to that date the organisms now called coccidia were generally known as "psorosperms"; whilst the condition of being infected with them—now called coccidiosis—was described as "psorospermosis[1]." The name "psorosperm" was introduced by Johannes Müller, in 1841, to denote the spores of those remarkable organisms now called by Bütschli's name Myxosporidia; and the coccidia were generally distinguished from these as "oviform" or "egg-like" psorosperms, on account of their resemblance to the ova of many metazoa. It should be remembered, however, that "psorosperm" was a name often loosely applied not only to the coccidia but also to the spores of gregarines, and to other "Sporozoa" (Sarcosporidia, etc.), and for a long time it had no very precise meaning.

Gubler's case. The first recorded case of coccidiosis in a human being is probably that described by Gubler (1858, 1858 *a*)[2]. His patient was a quarryman aged 45 who died, apparently from peritonitis, in a Paris hospital. He suffered from digestive troubles, anaemia, and other disorders, and his liver was much enlarged. At the *post mortem* examination it was found to contain many tumours of cancerous appearance—some of them very large—in which were numerous "ovoid cells," which Gubler was disposed to regard as "the eggs of helminths." They are said to have been "at least four times larger" than the largest cells of the surrounding tissues, but no other indication of their size is given. As regards their structure, he says that "some of them were very regularly ovoid with a perfectly distinct double contour, and completely filled with a finely granular contents, the others more or less flattened, shrivelled, and apparently empty." The two ends of the "cells" were unlike—"one is rather blunter, the other shows a slight constriction...and ends with a small and somewhat flattened or even very slightly depressed surface, as though an operculum or micropyle were present." The protoplasm forming

[1] This term has survived until comparatively recently in medical literature, though it has long since vanished from zoology. Cf. Allbutt and Rolleston's *System of Medicine*, Vol. II, 1907, where the coccidia of man are reviewed under the heading "Psorospermosis."

[2] See also Davaine (1860), p. 263, who quotes Gubler's papers *in extenso*. So far as I have collated them, Gubler's two papers are identical in contents.

the contents was sometimes contracted from the wall. Gubler regarded these structures as probably the "eggs of a distoma," but emphasized their resemblance to the "eggs" found in the liver of the rabbit. His descriptions are unaccompanied by figures[1], but they appear to relate to bodies which certainly resembled the oocysts of *Eimeria stiedae*—the coccidian parasite of the rabbit's liver.

Gubler states that his observations were confirmed by Davaine, to whom he showed his "ovoid cells." Davaine (1860), however, while agreeing that the "oviform bodies" were "like the smaller variety[2] in the liver of the rabbit," adds that, at the time when he saw them, they were "unfortunately in an advanced state of decomposition" (p. 263). Leuckart (1863), who did not himself see Gubler's "cells," says that the case should probably be regarded as one of "so-called psorosperm formation"; and later (Leuckart, 1879) he definitely pronounced the bodies to be "Coccidia." He considered them to be the same as those which he himself recorded from other cases (*vide infra*). Leuckart (1879, p. 279) claims that he was the first to point out "the true nature" of Gubler's oviform bodies in 1863. It is true that he suggested (1863, p. 49, footnote) that they might be "psorosperms," and added later (Appendix, p. 740) that they certainly were "psorosperms." Nevertheless, his claim to have identified Gubler's bodies as coccidia in 1863 is not easily reconciled with his own statements at that date: for not only does he say elsewhere in this same work that "in man psorosperms have not been found with certainty" (p. 142), but he also says that, for his part, he is "most inclined to consider these [*i.e.* 'psorosperms'] as pathological tissue-elements" (p. 49, footnote). No doubt he forgot these statements afterwards (1879). Virchow (1860), who considered "psorosperms" to be "entozoic" in character, had previously expressed the opinion that Gubler's bodies were neither worm eggs nor psorosperms, but vacuolated cells such as he had found in cancerous growths.

It seems clear, therefore, that neither Gubler nor his contemporaries were able to identify with certainty the bodies which he had discovered. This can scarcely be wondered at, when it is remembered that very little indeed was known about the coccidia at that date, and the most divergent views were held about their nature. Later writers, however, have been unanimous in regarding Gubler's case as one of hepatic coccidiosis, and there is much to be said for this view.

Kjellberg's case. The first case of intestinal coccidiosis in man appears to have been discovered by a Dr Kjellberg, of Stockholm, in the Pathological

[1] Leuckart, in referring to Gubler's paper (1858), ends the reference with "mit Abbild." See Leuckart (1879), p. 279, footnote. There are no figures, however, and no references to any, in the copies of the *Mém. Soc. Biol.* or *Gaz. Méd. Paris* which I have consulted.

[2] Davaine (1860) considered that there were "two kinds of oviform corpuscle" in the liver of the rabbit. From his descriptions it appears that the "smaller variety" was merely a selection of the smaller-sized oocysts of *Eimeria stiedae* before their contents had undergone contraction and segmentation.

Institute at Berlin. The findings are recorded by Virchow (1860), who says that the parasites were found in the villi of the small intestine. Virchow adds that the "psorosperms" in this case "agree completely with those which I have seen in the dog." He describes the latter as "*paired* psorosperms" — "relatively small bodies, regularly arranged together in two's, provided with a strong double-contoured membrane." There can thus be little doubt that he had in mind the canine form of the coccidian parasite now generally known as *Isospora bigemina*. This supposition is strengthened by the fact that he further compares Kjellberg's "psorosperms" with similar bodies which he himself had found in the kidney of a bat, and of which he gives some figures. So far as I am aware, this parasite has never been studied or described since; but from Virchow's account, it seems probable that it was also a species of *Isospora* (=*Diplospora*).

It therefore seems probable that Kjellberg discovered, in the small intestine of man, a coccidian belonging to the genus *Isospora*, and closely resembling that of the dog (*I. bigemina*).

Virchow's case. Virchow (1860) further described and figured some bodies which he had himself discovered in the liver of a woman. They were somewhat large oval structures—some 75 microns in length[1]—with a thick shell surrounded by a gelatinous envelope, and with protoplasmic contents strongly suggesting helminth ova undergoing segmentation. Virchow regarded these bodies as "worm eggs," and suggested that they probably belonged to a pentastomid. He failed, however, to find any "worms" associated with them. Some later writers have pronounced Virchow's case to have been one of hepatic coccidiosis, believing the "eggs" to have been the oocysts of a coccidium. Railliet (1895), for example, says that Virchow's parasite was "quite clearly a coccidian" (p. 133); but I cannot find the slightest justification for such a statement. In my opinion the bodies found by Virchow were almost certainly not the oocysts of a coccidium, but were probably, as he believed, ova of a metazoan parasite. In any case, they seem to have been very different from the hepatic coccidia of Gubler, and cannot possibly have been the oocysts of *Eimeria stiedae*.

Eimer's cases. In his well-known work on coccidia, Eimer (1870) records the finding of "psorosperms" in two human bodies in the Pathological Institute in Berlin. The histories of the cases were almost unknown. "The alimentary canal was filled, and its epithelium completely infiltrated" with the parasites. Beyond noting that "the form of the 'psorosperms' was here the same as in the aforesaid animals" he gives no description of them. "The aforesaid animals," it may be noted, included mice and others, and the coccidia infesting them probably belonged to several different genera. In my opinion it is now quite impossible to identify Eimer's "psorosperms" with certainty.

[1] Virchow (1860) states that the length of the structures was 0·003 of a Paris inch. Blanchard (1889) and nearly all subsequent commentators give their length as 56 μ.

They were probably coccidia of some sort, though even this is open to question. Even their habitat cannot be exactly determined—in fact we know only that they occurred in some part of the gut ("Darmkanal"), and not in the liver.

Leuckart's cases. In his books on the animal parasites of man, Leuckart cites several cases of coccidiosis. These have long been copied from book to book, together with Leuckart's opinions on them, without criticism. As they have thus come to occupy an important place in the literature of this subject, I shall examine them in some detail.

It should be noted first that none of the cases cited by Leuckart was his own. They were all discovered and communicated to him by others. As noted above, he makes reference in the first edition of his book (Leuckart, 1863) to the "psorosperms" previously found in the human liver by Gubler. In the Appendix to this work (p. 740) he was able to add a second, and apparently similar case, communicated to him by a Dr Dressler of Prague. The case is mentioned by him again later (Leuckart, 1879, p. 281), and accompanied on both occasions by a woodcut from the sketches which Dressler had made of his findings. So far as I can determine, Leuckart himself never saw the actual specimens: he saw only the drawings which he reproduces. From the description and figures the following facts can be obtained. Dressler found three small nodules—varying in size from that of a millet seed to that of a pea—in the margin of the liver of a human cadaver. Nothing further is recorded about the case itself. Inside the nodules was a whitish pulp, containing oval bodies which were—to judge from the drawings—the unsegmented oocysts of a coccidian. Their length appears to have been about 18-20μ; otherwise they are not unlike the oocysts of *Eimeria stiedae* from the fresh liver of a rabbit. If we accept the statement that they were "psorosperms" or "coccidia," there is, nevertheless, no information in the descriptions which will enable us to determine the genus, let alone the species, to which they actually belonged.

The second case cited by Leuckart (1879, p. 281) was discovered by one Prof. Sattler of Vienna. A preparation, which he had made for the purposes of a course in pathological anatomy, was found to contain "a dilated bile-duct with greatly proliferated epithelium and coccidia" (Leuckart). Nothing appears to be known about the case from which the material was obtained. It is implied, though not definitely stated, that it was human. Sattler did not send the preparation to Leuckart, but to a Prof. Perls of Giessen, who sent it on to him, together with a drawing which is not reproduced. Leuckart says he was able to convince himself "of the true nature" of the coccidia by his examination of the specimen: but he notes that "the contents of the coccidia" had been "completely cleared" by mounting in glycerine, so that "the bodies in question could easily have been taken for distomum eggs (*D. lanceolatum*[1])."

[1] It is perhaps worth noting here that the eggs of this trematode (now known as *Dicrocoelium lanceatum*) are 38 μ to 45 μ in length or rather more than twice the length—that is, if his own figures are correct—of the "coccidia" which Leuckart says could be mistaken for them.

It must be confessed that the description of this case is far from satisfactory, and the identification of the "coccidia" now quite impossible.

Leuckart (1879, p. 282) gives yet a third case, "in which likewise our coccidia were demonstrable," which was also sent to him by Perls. It consisted of a preparation out of a collection made by von Sömmerring and later incorporated in that of the Pathological Institute at Giessen. No description is given either of the case or of the parasites, but Leuckart says that the specimen was labelled "*an Distomis orta.*" He believed this to be a corrupt reading of "*an Distomatis ova?.*" And it may be noted that at that date it was quite usual to regard the oocysts of coccidia as eggs of trematodes or other worms. Nevertheless, I cannot regard this case as anything but highly doubtful. Even if it be granted that the preparation contained coccidia, there is no proof that they were of human provenance. The origin of the preparation is unknown, its authenticity unvouched for, its label admittedly wrong. And it is far from improbable that the coccidia—if such they were—which it contained were those of the rabbit. For it is by no means unlikely that von Sömmerring, when engaged in collecting materials for a study of ulceration of the bile ducts, should have included in his collection specimens illustrating a condition so common in the rabbit and already well known at that date.

The three cases just described constitute the whole of Leuckart's contribution to the facts concerning human coccidiosis. Dressler's case, which he never saw, is, perhaps, the best attested. There is a very brief account of it, it was known to be a human case, and we have Dressler's own drawings of his findings from which to form an estimate of his parasites. Sattler's case, of which Leuckart saw a bad preparation and a drawing, and of which he learnt otherwise at third hand, is far more questionable. One may well hesitate to draw any definite conclusions from it. Finally, the case of Perls (von Sömmerring's preparation) has so little to support it, that, by itself, it is almost without value. If hepatic coccidiosis were a common condition in man, these last two cases might, perhaps, be less open to question. But when they constitute more than a third of the known, or alleged, cases on record, they require something more than Leuckart's loose statements for their substantiation.

Leuckart's opinions concerning the cases which he recorded are well known, and will be considered again later. It will suffice to note here that he regarded, without any apparent justification, all the hepatic coccidia of man as identical with those which occur in the liver of the rabbit.

Although it will be evident from what has just been said, I would here emphasize the fact that the *original* cases of human coccidiosis recorded by Leuckart are only three in number. Later authors have, apparently, been misled into supposing that there were four or even five[1]. I would also note

[1] The five cases sometimes given are those of (1) Dressler, (2) Perls, (3) Sattler, (4) von Sömmerring, (5) Leuckart. From the foregoing account it will be clear, I think, how this misconception has arisen.

that all were cases of *hepatic* infection—Leuckart himself having never studied or described any cases of intestinal coccidiosis in man.

Rivolta's cases. It is generally supposed that cases of human coccidiosis were studied and described by Rivolta, and his name is almost invariably cited as one of the authorities on the subject. The facts, however, appear to be as follows.

Rivolta (1873) mentions somewhat casually (p. 565) that he found some "corpuscles in the faeces" of "a man afflicted with intermittent fever"; and he gives (Plate X, fig. 317) some rude sketches of them, described (on p. 580) as "corpuscles in the faeces of a man suffering from tertian fever." The bodies in question were "large white cells, with somewhat indistinct contour and homogeneous whitish contents; they had an oval shape, some of them also being round or piriform" (p. 565). To judge from the figures, they were about 8μ in diameter: and although it is impossible to identify them, I may hazard the guess that they were the cysts of *Entamoeba nana*, which are far from uncommon in human faeces. Rivolta himself does not even suggest that his "corpuscles" were coccidia. So far as I can discover, he nowhere refers to them again; and in none other of his publications is there any mention of his having found coccidia—or any other protozoa—in the faeces or tissues of man. Grassi (1879), however, cites the above passage from Rivolta, and compares his findings with the supposed "Psorosperms" which he himself found in the faeces of human beings. But as we shall see (*vide infra*), Grassi's own "coccidia" were not coccidia at all, but the cysts of other protozoa.

In a later work, Rivolta (1878) proposes the name "*Cytospermium hominis*" for the "psorosperms" found in man by Eimer (1870). The name is proposed explicitly for these forms, and not for any organisms which he had himself studied or described. Its application turns, therefore, upon the identification of Eimer's parasites.

From a careful study of all the available sources of information, I have no longer any doubt that Rivolta neither discovered nor described any coccidial parasites of man, and that the cases attributed to him are due to a mistake which has become traditional. Its origin, I believe, is to be traced to Blanchard, who has been copied—without verification, and often with inaccuracy—by all later writers on human coccidiosis. But I shall return to this point later.

Grassi's cases. Grassi, like Rivolta, is almost invariably cited as a discoverer of coccidia in man; and, as I shall attempt to prove, with equally little justification.

As noted above, Grassi (1879) described some "corpuscles" which he had found in human faeces and which he regarded at the time as coccidia. They were found in two cases—a boy and a young man—in one of whom the infection was followed for some two-and-a-half months. From the brief description and figures in the original paper it is clear that the "corpuscles" were not coccidia at all, but probably the cysts of *Giardia* (= *Lamblia*) *in-*

testinalis and *Entamoeba coli*. This supposition is confirmed by Grassi's later writings. He there says definitely (Grassi, 1882, 1883) that the "special corpuscles" which he had previously described in human faeces were *not* coccidia. The larger forms were "resting amoebae"; of the identity of the smaller he is still in doubt. From his figures and description, however, it is certain that these were, for the most part, cysts of *Lamblia*, which Grassi and Schewiakoff (1888) identified and described at a later date.

It appears perfectly clear, therefore, that the "coccidia" discovered in human faeces by Grassi have no foundation on fact. Grassi himself, indeed, has denied that he ever found coccidia in man. He says (cf. Grassi, 1883, p. 442) that some of the "corpuscles" which he had earlier described were only "*perhaps related to* the coccidia": and he is indignant with Bütschli (1881) for having said that "their coccidial nature is still pretty doubtful." Bütschli, he says, had credited him with believing that they were coccidia in order to have the pleasure of making this retort. Nevertheless, there is good evidence that Grassi originally did believe that the "corpuscles" in question were coccidia[1], although he satisfied himself later that they were not. The matter is clinched by his own statement some years later that he "has never found intestinal coccidia in man" (Grassi, 1888, p. 5).

It is therefore certain, I think, both from his own testimony and from a careful consideration of his publications, that no real cases of human coccidiosis were ever discovered or described by Grassi.

Kunstler and Pitres's case. The case described by Kunstler and Pitres (1884, 1884 *a*) differs from all the others which we have to consider, in that the parasites were found, not in the liver, gut, or faeces, but in the pleural cavity. The patient was a man of 27 serving on board a ship plying between Bordeaux and Senegal. He entered hospital at the former place, suffering from pleurisy. About 2 litres of pus were evacuated from the pleural cavity by tapping. Examined microscopically, it was found to contain "a large number of ovoid or fusiform corpuscles,—homogeneous, of hyaline appearance, and swimming freely in the liquid of the preparation or enclosed, to the number of ten to twenty or more, in clear and relatively voluminous vesicles. The isolated corpuscles measure usually from 18 to 20μ in length; exceptionally, smaller (8 to 10μ) or much larger ones (60 to 100μ) are found." The "corpuscles" displayed a "finely striated envelope," and a central body believed to be a nucleus. In the opinion of their discoverers they were probably "the falciform bodies of psorosperms"—that is, the merozoites or sporozoites of a sporozoon —"living parasitically in the pleural cavity."

[1] This is not only implied by the title of his paper (Grassi, 1879), but is distinctly stated in it. After discussing the nature of his "corpuscles," he concludes by saying that he is "forced to the conviction that they are psorosperms." "Ed io li giudico psorospermi dell' uomo....I psorospermi da me descritti entrano nella sottofamiglia dei psorospermi oviformi-sferici" (p. 635). Allowing for the language of the period, he could hardly have expressed the opinion that they were coccidia with greater clearness.

The account given by Kunstler and Pitres, taken in conjunction with their illustrations[1], is to me extremely puzzling. I am unable to form any exact idea of the true systematic position of their parasite. I am by no means convinced that it was even a sporozoon of any sort—still less that it was a coccidian belonging to a familiar genus. This, however, was the view taken by Blanchard, who, in 1895[2], proposed the name "*Eimeria hominis*" for it. It is certain, at all events, that it cannot be placed in the genus *Eimeria* (Aimé Schneider, 1875) as now understood; and Blanchard's proposal was undoubtedly due to the imperfect knowledge of the coccidia prevailing at the time when he wrote. I conclude that "*Eimeria*" *hominis* Blanchard is certainly not an *Eimeria*, and that future work alone can show what the organism in question really was.

Podwyssozki's cases. Podwyssozki (1889) stated that he had observed four human cases of infection of the liver with "coccidia." The "parasites" live in the cells and their nuclei; they are difficult to find and recognize; and they are probably a cause of cirrhosis. "They attain the size of a considerable oval body, with a thick membrane, and containing one or several spores." The name "*Karyophagus hominis*" was first proposed for them (Podwyssozki, 1889); but later their discoverer stated that they "belong to the family of the Coccidia and probably to the *Coccidium oviforme*" (Podwyssozki, 1892). It is difficult, however, to recognize any resemblance to the coccidia of the rabbit's liver either in the descriptions or in the figures of these and similar structures published by Podwyssozki and Sawtschenko (1892).

I shall not consider these "parasites" further here. There is, I think, no longer any doubt that Podwyssozki's "coccidia"—like those described in cancerous growths by many others subsequently—were not coccidia at all, but cellular elements and cell-inclusions of various sorts which were mistaken for parasites.

Railliet and Lucet's cases. After redescribing the dog's coccidial parasite, originally called "*Cytospermium villorum intestinalium canis*" by Rivolta, Railliet and Lucet (1890) briefly record that they had discovered a similar organism in human beings. They had two cases—a woman and her child, who had both long suffered from chronic diarrhoea. The parasites were found in the faeces. They had a "regular ovoid form,"..."a certain number containing granular protoplasm with numerous refractile globules," whilst "others contained a large granular mass without these globules." Their mean length was 15μ, their breadth 10μ. The authors did not figure these

[1] Illustrations accompany their second paper (Kunstler and Pitres, 1884 *a*) only. The quotations given above are from their first paper (1884).

[2] I give this date on the authority of Blanchard himself (see Blanchard (1896, 1900) in bibliography). The first use of the name which I have been able to find is in his article in Bouchard's *Traité*, where he calls the organism "*Eimeria hominis* R. Blanchard, 1895," but gives no reference to the work of 1895 in which he proposed the name. I have not been able to find it. The second volume of Bouchard's work—in which Blanchard's account appears—is dated 1896.

bodies; nor did they, at this time, propose any name for them. They appear, however, to have regarded them as closely similar to the coccidia found in the intestinal villi of carnivores (dog, cat, polecat, etc.); that is to say, to the organism now generally known as *Isospora bigemina*. But they add that "the contents" of their human parasites "was sufficiently unlike" these.

A little later, Railliet and Lucet (1891) return to the coccidia of the Carnivora and accept the name then just bestowed upon them by Stiles (1891),—namely, *Coccidium bigeminum*. But they propose to subdivide the species into varieties corresponding to the different hosts in which the parasites had been found. The form in the dog they name *C. bigeminum* var. *canis*; that in the cat, var. *cati*; that in the polecat, var. *putorii*; and they add that there is perhaps a fourth, var. *hominis*, in man. Strangely enough, however, they make no mention of their own discovery in this connexion, but state that *hominis* "corresponds to the form observed by Kjellberg."

Railliet (1895), however, a few years afterwards, states that Kjellberg's parasite was probably *Coccidium bigeminum* (p. 146); and on another page (p. 140) he says that the forms found in human faeces by Railliet and Lucet probably belonged also to the same species.

It would appear, therefore, that Railliet and Lucet discovered two human cases of infection with an organism which they believed to be similar to *Isospora bigemina* of divers carnivores; that they identified it with the parasite previously found by Kjellberg; and that they proposed tentatively to name it *Coccidium bigeminum* var. *hominis*. The identity of Railliet and Lucet's parasites is, nevertheless, not beyond dispute. It is, in fact, impossible to recognize them as coccidia from their description alone. We have only the authors' opinions unsupported by any cogent facts; and the dimensions of their "coccidia" seem, at first sight, to contradict their hypothesis that the human parasites which they discovered could be a variety of *Isospora bigemina*. It is clear from their accounts, however, that—like other workers at this time—they did not understand the morphology of the "bigeminate" coccidia of carnivores. The two spores within the oocyst were regarded as two separate "coccidia"; and the measurements which they record relate, accordingly, in every case to the sporocysts—not the oocysts—of the parasites[1]. It is therefore possible that their human parasites, in spite of their apparently very small size, may really have been closely similar to *Isospora bigemina*.

Silcock's case. The only case of human hepatic coccidiosis described in England has been recorded by Silcock (1890). The patient was a woman aged 50 who died at St Mary's Hospital, London, in 1889. The chief symptoms noted before death were enlargement of the liver and spleen, fever, and slight diarrhoea. At the autopsy the liver was found to be "much enlarged," and to contain "a number of caseous foci arranged in groups," each of them surrounded by "a well-marked red ring of inflammatory congestion." The spleen

[1] See Railliet and Lucet (1890, 1891) and Railliet (1895).

showed "similar caseous foci." In the ileum were "six papule-like elevations with circumferential inflammatory zone," and in the large intestine "deeply congested patches of mucous membrane from 1 to 3 inches square." The caseous nodules—superficially resembling tuberculous lesions—"consisted largely of agglomerations of small, oval, egg-like bodies, with granular contents and well-marked capsule." The bodies were found "in the liver cells and in the bile ducts," and Silcock states that they were "identical in every respect with the coccidia described" by Leuckart "in cases of parasiticism (*sic*) in man and rabbits by the *Coccidium oviforme*." "On cultivating the coccidia...by keeping small affected portions of the organs in water... psorosperms were freely developed." He remarks that he has nothing else to add to Leuckart's description of the organisms, and gives no figures. It may be noted that Silcock's coccidia were apparently present in the liver *and in the spleen*—a remarkable situation. But although lesions are also mentioned in the gut, it is not explicitly stated whether similar parasites were found in them or not.

From Silcock's imperfect account it is now impossible to ascertain what he really saw. He seems, most unfortunately, to have thought that Leuckart had already fully investigated the hepatic coccidia of man—as was by no means the case. It seems clear that Silcock's coccidia resembled *Eimeria stiedae*, since he specifically mentions "*Coccidium oviforme*." But he gives no indication of their size, and does not appear to have noticed that the oocysts of the human coccidium figured in Leuckart are only about half the size of those from the rabbit. Moreover, Silcock himself was—all unawares—actually the first to study the development of the spores within the oocyst of the human parasite[1]; and it is most regrettable that he makes no mention of their number, form, or contents, since it is upon these that the identity of the parasite turns. Of the human parasite Leuckart saw nothing but the unsegmented oocysts, or drawings of them. His figures of "*Coccidium oviforme*" relate exclusively to the parasite of the rabbit; and his assumption that the two forms develop in the same manner, and are, in fact, the same species, is quite gratuitous. Silcock gives no reference to the work of Leuckart which he consulted, and he obviously cannot have studied his writings with any care. Had he done so, he might have been able to add much to our knowledge: but as it is, we can only guess what he really found.

Giles's cases[2]. In a work dealing with kala-azar and ankylostomiasis in Assam, Giles (1890) records the finding of numerous "coccidial" infections among the natives. Examination of his figures and descriptions leaves no doubt in my mind that his "coccidia" were really vegetable cell débris in the faeces of his patients. They were often present in "astonishing" numbers.

[1] Or, as he termed it, the development of "psorosperms" in the "coccidia" on "cultivation."

[2] I have here to thank my friends Dr A. C. Stevenson and Dr G. C. Low—the former for directing my attention to Giles's observations, the latter for obtaining his publication for me.

"They vary a great deal in size," but are "generally very much larger than the ova" of *Ankylostoma*..."averaging about $\frac{1}{150}$ inch [about 170μ] in diameter." "They are by no means uniform in shape...differing in fact from ova much in the same way that potatoes do from eggs." And so forth.

It will be sufficient for our present purpose to note that Giles's bodies, whatever else they may have been, were certainly not coccidia.

Jürgens's case. At the *post mortem* examination of a workman, aged 66, Jürgens (1895) discovered some peculiar "bodies" in growths in the *dura mater* of the brain and spinal cord. One of the rabbits which he inoculated from these subsequently developed numerous tumours—in the orbit, and various other places—which were believed to contain similar bodies. For reasons which are not apparent, the author regarded the bodies as a "species of coccidium"; but he added that "as regards size, shape, and sporulation it differs essentially from *Coccidium oviforme*, or *perforans*." Most people, on reading his account, will, I think, be disposed to go further than this; and they will probably conclude, as I do, that his "bodies" differed materially from all known coccidia, and consequently that his case was not one of coccidiosis.

Quincke's cases. Quincke (1899) has described bodies which he found in the faeces of two human beings, and which he regarded as coccidia. His first case (Case I), a man of 40, suffering from diarrhoea, was passing numerous "hyaline, oval structures, 12μ long, 8μ broad." These Quincke considered to be coccidia similar to those described by "Baillet" and Lucet, Grassi, and Rivolta. (What these authors actually described we have already noted.) From the description and figures, there can be very little doubt that Quincke's "coccidia" really were identical with those described by Grassi; that is, they were the cysts of *Lamblia intestinalis*, and not coccidia at all.

Quincke's second case (Case III) passed different bodies, which it is suggested were possibly coccidia. They were undoubtedly in reality *Blastocystis hominis*. The figures unmistakably depict this common vegetable organism of the human bowel.

It is thus clear that neither of the cases described by Quincke was one of coccidial infection.

Thomas's case. In the brain of a woman, aged 40, who died of pneumonia, Thomas (1899) found a bony tumour the size of a small pea. Microscopic examination of this led him to conclude that it had been caused by the presence of coccidia—which he identified as "*Coccidia oviformia*"—in the brain. The occurrence of the hepatic coccidia of the rabbit in the human brain, however, would be so remarkable a phenomenon, that it would require much stronger evidence for its verification than the author appears to have been able to adduce[1]. It seems, indeed, more than probable that Thomas was mistaken in his conclusions, and that the structures which he observed were not coccidia.

[1] I have not been able to consult the full account of this case, which was to have been published —according to the abstract (by Nuttall) in the *Centralbl. f. Bakt.*—in the Boston Hospital Reports.

Grunow's case. Grunow (1901) has described at length a case of enteritis believed to be due to protozoa—probably coccidia. The patient, a workman aged 61, had suffered for some time from diarrhoea. In his stools Grunow found round "bodies" about 6μ to 8μ in diameter, but sometimes larger. These, for reasons which are not apparent, were regarded as probably coccidia. From the description it is not possible to identify them. At the *post mortem* examination the small intestine was found to be ulcerated, and in sections of it the author succeeded in finding in the villi some other "bodies" which he was likewise disposed to regard as coccidia. The size of these is given as "4·9–7·3 broad, 7·3–9·8 long" (presumably microns). Although there was nothing to prove that the bodies in the faeces were in any way connected with those in the villi, the author united them by the following curious argument: "The parasites found in the faeces and in the mucous membrane are indeed different in form, nevertheless identical in their parasitic nature." He concludes by deciding that they are all probably coccidia, and *if so*, then *Coccidium bigeminum*. Comment is needless.

It is unnecessary to discuss this case in greater detail. There is not even the slenderest evidence that the various "bodies" in question were even protozoa; and probably nobody acquainted with these organisms will, after reading Grunow's account, be inclined to agree with him that his case was one of coccidiosis.

To the foregoing cases I will add, for the sake of completeness, a few notes on certain others which are now generally recognized as not coccidial, though at one time held to be such. The most important of these are the cases of infection with the two organisms now known as *Coccidioides* and *Rhinosporidium*: the rest are the very numerous cases in which "pseudo-coccidia" have been described and causally connected with divers diseases.

Coccidioides. This organism is now known to be a fungus. It invades the skin, central nervous system, and other organs, giving rise to the disease generally known as "coccidioidal granuloma." Some fifty cases are known, mostly from California. The parasite was discovered in S. America by Posadas, and considered by him and Wernicke (1892) to be a "coccidium." Rixford and Gilchrist (1896), who subsequently studied the parasite and the disease in detail, regarded the organism as a "protozoon," of doubtful systematic position, and introduced the name *Coccidioides immitis*[1] for it—a name suggested by Stiles. It has since been cultivated by Wolbach (1904) and others, and appears to belong to the Oomycetes[2].

[1] Hartmann (1912), in ignorance of the fact that the organism was already well known and named, has more recently proposed the name "*Blastosporidium schooi*" for it, believing it to be a "Haplosporidian."

[2] For further information and literature concerning this parasite, see—in addition to the authors cited above—Evans (1909) and Dickson (1915).

Rhinosporidium[1]. This curious organism, which gives rise to polypoid growths in the nose, appears to have been discovered by Malbran in S. America in 1892. Another case was found there by Seeber in 1896, and first described by him in 1900. O'Kinealy independently discovered the organism in Calcutta in 1894, but did not describe it until 1903. Since then other cases have been studied, mostly from India. Wernicke regarded the parasite as a coccidian, and in 1900 named it *Coccidium seeberi*. Minchin and Fantham (1905), in ignorance of Seeber's work, restudied the organisms from O'Kinealy's case and named them *Rhinosporidium kinealyi*. They regarded them as "Haplosporidia." Although the systematic position of *Rhinosporidium* is still very problematic, it may be stated with some confidence that it is almost certainly not a coccidium[2]. I may add that to refer any organism to the group Haplosporidia practically amounts, at the present moment, to a confession of ignorance of its true status.

Pseudo-coccidia. In addition to the highly questionable "organisms" which we have already had occasion to notice, "pseudo-coccidia"—bodies of various sorts mistaken for coccidia, "psorosperms," or related protozoa—have been described from almost every organ and part of the human body. Many of these have been found in malignant growths, and have excited a transient interest, and occasionally attained some celebrity, as "causes" of cancer. The cases of Podwyssozki, and his "*Karyophagus hominis*," have already been noticed. They will serve as an example of a class of cases and parasites too numerous to mention, and now of nothing but slight historic interest. The literature concerning these and similar "parasites" has been collected by Labbé (1899), whose work should be consulted by those interested in the subject.

Of other pseudo-coccidia it will suffice to mention a few of the best known. The oldest historically are those discovered by Lindemann (1863, 1865), who was one of the first to find "psorosperms" and "gregarines" in man. He recorded their presence in the heart, aortic valves, and kidney; and did not hesitate to identify the forms in the heart-muscle with the coccidia of the rabbit's liver—*Eimeria stiedae*—which he regarded as a *Monocystis* (cf. Lindemann (1865), p. 385). The value of his observations may be gauged from the fact that he also recorded the discovery of "gregarines" lying on the surface of human hairs—an observation at first accepted, but later justly censured by Leuckart (1879). Similar "coccidia" discovered in the kidneys and generative organs—especially of the female[3]—by later investigators, deserve no further notice here.

[1] For the facts, names, and dates given in this paragraph, see Seeber (1912) and Minchin and Fantham (1905). From Seeber's account it appears that the correct name of the organism is *Rhinosporidium seeberi* Wernicke, 1900.

[2] On this point the views of Seeber and Minchin are in agreement. It is also the opinion which I have formed from the examination of some excellent preparations which my friend Dr J. H. Ashworth of Edinburgh has very kindly shown me.

[3] Cf. Pick (1895): "Our knowledge of the Sporozoa in gynaecology must, at the present time, be described as entirely negative."

The "coccidia" found in the skin in Darier's disease ("*psorospermosis follicularis vegetans*"), in Paget's disease of the nipple, and in other affections, by Darier (1889) and others, are now generally recognized as modified epidermal cells (cf. Boeck (1891), etc.). The "coccidia" of *molluscum contagiosum* are probably of a similar nature[1]. These and similar diseases, once included among the "psorospermoses," have long ceased to be regarded as the results of coccidial infection.

In the preceding paragraphs I have enumerated all the cases of human coccidiosis which, so far as I have been able to ascertain, had been put on record prior to the year 1915. I have omitted to consider in detail those cases only which, by general consent, have already been finally consigned to oblivion. It will be convenient at this point, before going further, to summarize briefly the main conclusions which may be drawn from a study of these cases. I will therefore do so: but I would point out that these conclusions are drawn entirely from the facts recorded by the original describers of the various organisms concerned, and independently of the opinions of later commentators which will be considered in the next section.

(1) There is good evidence that at least nine of the authors who have described, or who are alleged to have described, coccidia from man, did not, in fact, do so. The organisms or structures which they found were certainly not coccidia. These authors are: Virchow (1860), Rivolta (1873, 1878), Grassi (1879), Podwyssozki (1889), Giles (1890), Jürgens (1895), Quincke (1899), Thomas (1899), Grunow (1901). The cases of human coccidiosis attributed to these workers should therefore be eliminated from the literature of the subject. To this *index expurgatorius* must also be added, of course, the names of all the describers of "pseudo-coccidia"; and for my own part, I am disposed to add also the names of Kunstler and Pitres (1884). Despite the authority of Blanchard, I cannot regard theirs as anything but a most questionable case of coccidiosis.

(2) This leaves us with only six works which record cases of infection with organisms that can be regarded, with different degrees of certainty, as coccidia. The parasites were recorded from the liver, intestine, or faeces, as shown in the following synopsis:

In the *liver*, by

Gubler (1858)	1 case
Dressler [recorded by Leuckart (1863, 1879)]	1 „
Sattler and Perls [recorded by Leuckart (1879)]	1 „
Perls and v. Sömmerring (?) [recorded by Leuckart (1879)]	1 „
Silcock (1890)	1 „
Carried forward	5 cases

[1] For the literature see Labbé (1899) and Lipschütz (1912).

Brought forward	5 cases
In the *intestine*, by	
Kjellberg [recorded by Virchow (1860)]	1 case
Eimer (1870)	2 cases
In the *faeces*, by	
Railliet and Lucet (1890)	2 ,,
Total ...	10 cases

From this it may be concluded that human coccidiosis is not a common affection: especially when it is remembered that several of the above cases are, to say the least, by no means well authenticated[1].

(3) As regards the parasites present in these cases, we find the information necessary for their identification either wholly lacking or exceedingly deficient. The coccidium inhabiting the liver appears to resemble *Eimeria stiedae* of the rabbit. There is no evidence, however, to prove that it is of this species; and there is some—namely, its size—to indicate that it is not. The coccidia found in the small intestine and in the faeces were, so far as there are any indications, of a species belonging to the genus *Isospora*, and resembling *I. bigemina* of cats and dogs. In short, there is evidence that man may harbour an *Eimeria* in his liver and an *Isospora* in his gut; but beyond this it would be hazardous to draw any inferences.

The foregoing conclusions may be fairly drawn, I think, from the cases analysed in the present section. At the same time, however, it must be pointed out that the cases considered—or at least some of them—appear less uncertain when viewed in the light of more recent work. The conclusions are here tentative, not final. They constitute merely a "*vindemiatio prima*," in the Baconian manner, before we proceed to study further instances. But it is well to state them clearly at this point, since they give us a basis of fact from which to judge the value of the opinions which have been expressed at various times by writers on human coccidiosis. The most important of these opinions I shall now chronicle as briefly as possible.

B. Opinions concerning the Coccidial Parasites of Man, as expressed by the Chief Compilers and Commentators.

Various writers have, on various occasions, held widely different views regarding the coccidia parasitic in man. These differences—so far as concerns us at present—are chiefly regarding (1) their systematic position; (2) their specific identity or diversity in respect to similar parasites occurring in other hosts; and, consequently, (3) their nomenclature. Even the most casual con-

[1] For my own part, I am quite prepared to believe that the cases of Gubler, Dressler, Silcock, and Kjellberg, are the *only* real cases of human coccidiosis recorded. But such a view is, perhaps, hypercritical.

sultation of the literature on this subject will show that, in each of these respects, the view which any writer takes of the coccidia of man depends very largely upon the opinions which he holds concerning the coccidia parasitic in rabbits, and those of dogs, cats, and other carnivores. Until recently there has been much uncertainty about these parasites; and it will greatly facilitate the comprehension of what I have to say in the ensuing paragraphs if the present state of our knowledge of them is made clear at the outset. I shall therefore devote a short space to the consideration of this subject before proceeding further.

The coccidia of the rabbit. As is generally known, the common rabbit, both wild and domesticated, usually harbours coccidia in its liver and small intestine. In the liver they infest the bile canal system, in the gut the epithelium of the mucous membrane; and in both situations they may give rise to pathological conditions of variable and sometimes fatal extent.

The coccidia of the liver were discovered by T. G. Hake, a London physician, in 1839[1]. He did not, however, understand their true nature; but regarded the lesions in the liver as "carcinoma," and the parasites as "a new form of the pus globule." As a result, however, of the labours of many later workers in several different countries—it will suffice to mention Remak, Kölliker, Stieda, Lieberkühn, Rivolta, Leuckart, and Balbiani—the parasites gradually came to be regarded in their proper light; so that by about 1880 they were generally recognized as protozoa belonging to the group of "oviform psorosperms" or Coccidia. Hake (1839) had already found oocysts in the contents of the duodenum, and later Klebs (1859) demonstrated the parasites within the epithelial cells of its mucous membrane. Since then there has been a prolonged controversy over these parasites, and even now it cannot be regarded as finally decided. The majority of modern workers follow Balbiani (1884) in regarding the coccidia found in the liver and the intestine as belonging to the same species—the site in the host's tissues being their sole distinctive character. Others, adopting the standpoint of Leuckart (1879), regard the parasites of the intestine as specifically different from those in the liver, and advance various reasons in support of their belief. To my mind, the evidence at present available, both morphological and experimental[2], is undoubtedly in favour of the

[1] It may be noted that Hake's work contains the earliest account and figures of coccidia known. His drawings of the oocysts from the liver are admirable, and might serve as illustrations for a modern text-book.

[2] The most important experimental evidence is that adduced by Railliet and Lucet (1891), Railliet (1895), and Lucet (1913). According to these workers the spores of the hepatic parasite, when ingested, produce a hepatic infection only: and similarly, those of the intestinal parasite produce an intestinal infection only. If the work of Lucet (1913) is sound, as there appears no reason to doubt, then it seems certain that his conclusions are correct—namely, that there are two distinct species in the rabbit. So far as my own observations enable me to judge, the morphological differences which he describes between these species actually exist, and at present I agree with his conclusions. Moreover, I can find no adequate arguments against his views in the work of Metzner (1903), Reich (1913), and others who hold an opposite opinion.

latter view, which is that of some of the modern French writers. The former, which regards all the coccidia in the rabbit as belonging to one and the same species, is, however, that which has gained general currency in England and Germany.

The parasites of the liver and the gut both unquestionably belong to the genus *Eimeria* Aimé Schneider (1875). The correct name of the former is *Eimeria stiedae* Lindemann (1865)[1], as was pointed out by Stiles (1902) and Lühe (1902); but it has been given several other names, of which the most important are *Psorospermium cuniculi* Rivolta (1878), and *Coccidium oviforme* Leuckart (1879). In all but the most modern works it appears under the names *Coccidium cuniculi* or *C. oviforme*.

The correct name of the coccidium of the rabbit's small intestine—assuming it to be a separate species—is *Eimeria perforans* Leuckart (1879). Those who regard the two forms as identical apply the name—whatever it may be—which they give to the hepatic form, to the intestinal form also, as the former was named before the latter. In many modern works, therefore, *E. perforans* appears as a synonym under *E. stiedae*. In the older writers it figures either independently as *Coccidium perforans* or as merely the intestinal form of "*C. oviforme*" or "*C. cuniculi*."

The intestinal coccidia of carnivores. The coccidia living in the intestinal villi of cats, dogs, and other carnivores, have also a somewhat chequered history. They were first seen in the dog by Finck (1854), who regarded them as metabolic products. Later workers—Virchow, Rivolta, Grassi, and others—recognized their parasitic character, and showed that they occur in the cat also[2]. The chief features in their development have been made out by Grassi (1879 *a*), Railliet and Lucet (1890, 1891), Stiles (1891, 1892), and more recently by Swellengrebel (1914).

Rivolta called the parasites "oviform cells" or "*Cytospermia*" of the villi of the dog and cat[3]. They were subsequently named *Coccidium Rivolta* by Grassi (1879 *a*) and *Coccidium bigeminum* by Stiles (1891). They certainly do not belong to the genus *Coccidium* (Leuckart, 1879), which is a synonym of *Eimeria* (Aimé Schneider, 1875); and as they are characterized by possessing an oocyst containing two tetrazoic spores, they are now generally referred to Schneider's genus *Isospora*, and known as *Isospora bigemina* Stiles. It has not been determined with certainty whether the forms inhabiting different hosts belong really to the same or to different species. Most authors, however, follow Railliet and Lucet (1891) in considering the parasites occurring in different

[1] Lindemann, it may be noted, called the organism *Monocystis stiedae*, and regarded it as a gregarine.

[2] In both these hosts they are far from uncommon. Similar—possibly identical—forms have also been described in the pole-cat (*Mustela putorius* L.) and wild species of dog.

[3] "Cellule oviformi dei villi del cane e del gatto" (Rivolta, 1874, 1877, 1877 *a*); "*Cytospermium villorum intestinalium canis*" (Rivolta, 1878).

animals as varieties of the same species. In most works dealing with the Coccidia they are called collectively "*Coccidium bigeminum*[1]."

Having said so much by way of introduction to this section, I will now pass on to its subject proper. I shall take the various authors in chronological order, as far as this is possible.

It has already been noted that Davaine (1860) saw Gubler's hepatic coccidia, and tentatively concluded that they were similar to those found in rabbits (*i.e.* to *Eimeria stiedae*). He called all these parasites "oviform bodies," and was inclined to regard them as the eggs of worms.

Of much greater importance historically are the opinions of Leuckart. As already noted, he first expressed the opinion that Gubler's case was "of doubtful nature." He included with it Virchow's and Kjellberg's cases (Leuckart, 1863, p. 49, footnote). Later, however, he decided that Gubler's case was one of "psorosperm" infection, and similar to Dressler's case and others which he had since had communicated to him (Leuckart, 1863; Appendix, p. 740). In his later work (Leuckart, 1879) he discusses all the parasites in greater detail. Translated into modern terms, his opinions were as follows: all the coccidia found in the human liver[2] are of the same species as those found in a like situation in the rabbit. In other words, all are *Eimeria stiedae* (= *Coccidium oviforme*). All the coccidia found in the intestine of man[3], the rabbit, the cat, and the dog, also belong to one species—namely, *Eimeria perforans* (= *Coccidium perforans*). This species therefore embraced *Isospora bigemina* in Leuckart's estimation.

Leuckart believed that human beings acquire their infections through contact with infected animals. He thus regarded the rabbit as a source of human contagion, for both the hepatic and intestinal coccidia; and for the latter, the

[1] Although the organisms in question are now very generally known as *Isospora bigemina* Stiles, it seems probable that this is not their correct name. If we exclude Rivolta's lengthy Latin appellation (see preceding footnote) as not conforming with the rules of binominal nomenclature, the first name given to them seems to be "*Coccidium Rivolta*," bestowed by Grassi (1879 *a*). Why the specific name is written in the nominative instead of in the genitive case is not clear. At first sight it appears to be a *lapsus calami*; but Grassi repeats the name in the same form in at least two later papers (Grassi, 1882, 1883). It is clear, however, that he regarded the name as binominal, and intended it as a mark of honour to Rivolta. My own view is that this name should be amended by substituting the correct generic name, and putting "*Rivolta*" in the genitive case. The correct name would then be *Isospora rivoltae* Grassi (1879), which has priority over *I. bigemina* Stiles (1891). If anybody should object to this specific name on the grounds of Art. 14 of the International Rules, I would remark that the genitive termination *-ae* is good Latin for the name of a man ending in *-a* (*e.g. Caligula, -ae*; *Cotta, -ae*, etc.) in spite of the Rules; and further, that if *Eimeria stiedae* is an acceptable form of commemorating the Russian naturalist, then *Isospora rivoltae* should be equally acceptable for the Italian. Forms such as "*rivoltai*" appear to me to be unnecessary and objectionable solecisms. The difficulties connected with the generic name *Isospora* will be considered later. To avoid confusion, I shall, however, continue to call the coccidia of the cat and dog *Isospora bigemina* in the present paper.

[2] Leuckart (1879) knew of four cases—Gubler's, Dressler's, and the two cases sent by Perls (from Sattler and the von Sömmerring collection).

[3] The only human cases known to Leuckart were those of Kjellberg and Eimer.

cat and dog also. If we seek his grounds for this belief, they are difficult to find. In fact his opinion appears to rest mainly upon a general belief that there are only a few species of coccidia. As evidence that the hepatic coccidia of man and rabbit are the same, he advances the argument that Gubler's case probably acquired his infection from a rabbit; for the singular reason that he lived in France, and France is well known to be the home of rabbit-breeding[1]. It is to be noted that neither as regards Gubler's case nor any of the other recorded cases of human hepatic coccidiosis, is there even the slightest evidence of the patient's previous contact with rabbits.

Evidence for his belief in the identity of the intestinal coccidia of man, the rabbit, the dog, and the cat, Leuckart has none, beyond the fact that all the parasites occupy a similar site in their hosts.

It is only fair to acknowledge that Leuckart could hardly be expected to hold correct views concerning the Coccidia, since our knowledge of these organisms—and, in fact, of all the Sporozoa—was both deficient and inaccurate at the time when he wrote. Nevertheless, it is somewhat surprising that his baseless speculations should have been treated with the respect and approval which they have very generally received from later workers.

Rivolta (1873), who had himself studied the coccidia of the rabbit, merely notes the occurrence of similar "psorosperms" in the cat and in man[2]. He does not express any opinions regarding them. After a further study of *Isospora bigemina*[3], however, he attempted a classification of all the coccidia known to him (Rivolta, 1878). He regarded the coccidia of man[4] as distinct from *Eimeria stiedae* and *Isospora bigemina*, and named them *Cytospermium hominis*. He placed *I. bigemina* in the same genus ("*Cytospermium*"), but referred the rabbit coccidia to a different one ("*Psorospermium*"). Although no definitions of his genera are to be found either here or elsewhere in his works, his general conception of the relations of these forms to one another was thus, as far as it went, approximately correct.

Bütschli (1882), in his great treatise on the Protozoa, deals with the early history of the investigation of the Coccidia. He refers to the human cases of infection then known, but does not attempt to identify or classify the parasites. Somewhat later, Balbiani (1884) traverses the same ground. He also alludes to the coccidia found in man, and says they are supposed to be the same as those of animals (rabbits, etc.); but of this he seems doubtful.

Blanchard (1889) deals with the coccidia of man in some detail. He cites correctly the cases of hepatic infection recorded by Gubler and Leuckart, and regards the parasites present in all these as "*Coccidium oviforme*"—*i.e.* as

[1] "In Frankreich ist bekanntlich die Kaninchenzucht mehr als sonst irgendwo zu Hause" (Leuckart, 1879, p. 280). He then proceeds to draw a fanciful picture of how Gubler's case, "with a certain probability," acquired his infection—by drinking water from an imaginary cistern contaminated by proximity to a hypothetic rabbit-hutch.

[2] He refers only to Gubler's case (*vide* Rivolta (1873), p. 382).

[3] See Rivolta (1877, 1877 *a*).

[4] The only cases which he mentions are those of Eimer (1870).

Eimeria stiedae of the rabbit. Virchow's case[1] he regards as "analogous." He notes, however, for the first time, that there is a difficulty involved in this supposition: for the oocysts from Dressler's case, as figured in Leuckart, appear to be only about half the length of those found in the liver of the rabbit; whilst the structures described by Virchow were nearly twice as long as the latter. (See Blanchard (1889), p. 48.)

The coccidia found in the human intestine by Kjellberg and Eimer are regarded by Blanchard as "*Coccidium perforans*" (*i.e.* as *Eimeria perforans* of the rabbit's gut). He thus separates them from *Isospora bigemina* (= "*Coccidium Rivolta* Grassi, 1881") of the cat. Under the human cases of infection with *E. perforans* he also cites the findings of Rivolta and Grassi. He says: "Rivolta was able to observe coccidia, in the living state, in the excrement of children and adults: in a young boy he found, during nearly three months, that the coccidia were discharged with the dejecta. He found them also in a man afflicted with intermittent fever, and Grassi has published analogous observations" (Blanchard (1889), p. 48). As I have previously pointed out, neither Rivolta nor Grassi really found coccidia in human faeces; and moreover, it seems clear that Blanchard confused with one another the observations of these two workers[2]. Rivolta's "children and adults" appear to be the boy and the young man studied by Grassi (1879), and these are presumably the "analogous" cases which the latter is said to have observed. I have cited this passage from Blanchard *in extenso* because it seems to me to have been largely responsible for the erroneous statement, which has been so frequently repeated, that Rivolta and Grassi discovered coccidia in the faeces of human beings.

In a later work Blanchard (1896) expresses the same views, but with some change of nomenclature. He still considers the human hepatic coccidia to be the same as those in the rabbit, but calls them "*Coccidium cuniculi* (Rivolta, 1878)." As human cases he cites those of Gubler, Perls, Sattler, and Silcock. He also still considers most of the intestinal coccidia of man to be identical with the intestinal parasites of the rabbit: but he now names all of them—from both hosts—"*Coccidium hominis* (Rivolta, 1878)." The human cases given are Eimer's together with the alleged cases of Rivolta and Grassi once more. He adds Railliet and Lucet's two cases to these, but regards them as doubtful because the site of infection was not ascertained. On the present occasion, however, he says that *Isospora bigemina* ("*Coccidium bigeminum* Wardell Stiles, 1891") also probably occurs in man. The only human case which he refers to this species is Kjellberg's. The organisms described by Kunstler and Pitres are called "*Eimeria hominis* R. Blanchard, 1895," and

[1] That is, Virchow's (1860) own case—not the case of Kjellberg recorded by him.

[2] Blanchard does not specify the works in which he found these observations. The only bibliographic reference to Rivolta is to the second edition (1884) of his "*Parassiti vegetali*," which I have not been able to consult. It is possible that this differs from the first (Rivolta, 1873), which I have consulted; but from the fact that both contain the same number of pages and plates (according to Stiles and Hassall's *Index-Catalogue*) this does not seem to me very probable. The explanation of Blanchard's erroneous statement is, I believe, that given above.

thus included among the coccidia. For reasons which are not given he now regards Virchow's parasites ("pentastome eggs") as "more or less related" to this form.

Blanchard's (1900) more recent account of the coccidia of man is essentially a repetition of that just summarized (Blanchard, 1896).

In his two later works Blanchard makes no reference to his earlier doubts regarding the identity of the hepatic coccidia of man and rabbit. He appears to agree with Leuckart in regarding all the coccidia of man as identical with those found in other animals. The chief differences between his earlier and later works, and between his and Leuckart's, are merely differences in nomenclature. I have considered these two authors at some length, for their opinions have influenced all later writers—most of whom appear, indeed, to have copied them without verification or criticism.

L. Pfeiffer (1891), in his work on the parasitic Protozoa, mentions the Coccidia of man. The human cases of coccidiosis are, he says (p. 56), given in Leuckart's works: but he records (p. 57) that the "cysts" of the human parasite have a size ("Masse") of "56 mmm.," citing as his authority one "Dechsler," to whose work he gives no reference. The only explanation which I can suggest of this statement is that Pfeiffer confounded Dressler's case (= "Dechsler's"?), given in Leuckart, with the measurements of Virchow's parasites, as estimated by Blanchard (1889). If we may judge from the rest of his book, a mistake of this sort might quite well have been made by this author. It may be added that he apparently regarded *E. stiedae*, *E. perforans*, and all the coccidia found in man—both hepatic and intestinal—as belonging to one and the same species.

In his well-known ***Treatise***, Railliet (1895) gives an account of the coccidia of man essentially similar to that of Blanchard. He says *Eimeria stiedae*[1] "can invade the human liver, as the observations of Gubler, Dressler, Perls and Sattler, von Sommering (Leuckart), Silcock, etc.[2] show" (p. 138). *Eimeria perforans*[3], he says, develops in the intestinal epithelium of the rabbit and man (p. 138); and as human cases he gives Eimer's. He says further that "various observers, among others Grassi, Rivolta, Railliet and Lucet," found coccidia in human faeces. Their site of infection is unknown, and the species to which they belong therefore uncertain: but he considers that the patients studied by himself and Lucet[4] were probably infected with *Isospora bigemina*[5] (p. 140), and that Kjellberg's coccidia were also probably of this species (p. 146). Virchow's own case, he says, is "quite clearly a coccidium," and probably an *Eimeria*, as also is the organism described by Kunstler and Pitres (p. 133). As we have already noted, this is not at all obvious: in fact, I am

[1] "*Coccidium oviforme* Leuckart = *C. cuniculi* Rivolta."

[2] I do not know who these others are supposed to be, since Railliet appears to have named all the known cases already.

[3] "*Coccidium perforans* Leuckart = *C. hominis* Rivolta."

[4] Railliet and Lucet (1890).

[5] "*Coccidium bigeminum* Stiles."

unable to see any real resemblance between these two organisms and any *Eimeria* with which I am acquainted.

Labbé (1896), in his first systematic account of the Coccidia, mentions briefly the forms recorded from man. He appears to regard them all as belonging to one species[1], which he calls "*Coccidium hominis* Rivolta"; but he says they "strongly resemble *C. perforans* and *C. bigeminum*," which—as also *E. stiedae*—he accepts as distinct species. As describers of "*C. hominis*" he cites Eimer, Rivolta, Leuckart, Virchow (Kjellberg), and Railliet. The organisms of Kunstler and Pitres he considers very doubtful. In his later and more extensive work (Labbé, 1899) he appears to have changed his opinions: for we here find the hepatic coccidia of man referred doubtfully to *Eimeria stiedae* ("? *Coccidium cuniculi* Rivolta"), whilst the forms from the human intestine are regarded as a variety of the species occurring in the same situation in the rabbit. He calls them "*Coccidium perforans* var. Kjellberg," and enumerates the cases of Kjellberg, Eimer, "Grassi, 1880," and Railliet and Lucet, under this heading.

Doflein (1901), and even later in the third edition of his text-book (1911), appears to believe that the hepatic and intestinal coccidia of man both belong to the same species as that occurring in the rabbit's liver (*E. stiedae*). Infection of the human liver with this species has been, he says, "observed in repeated cases," but he cites no authorities. He notes further that "some cases of coccidiosis in man" may be due to infection with *Isospora bigemina*.

The account of the coccidia of man given by Braun (1903, 1906), seems to be copied, with a few minor changes and additions, from Blanchard (1896, 1900). The human hepatic coccidia are regarded as identical with those of the rabbit ("*Coccidium cuniculi* Rivolta, 1878"—that is, *Eimeria stiedae*). He cites the cases of Gubler and Silcock, and those recorded by Leuckart[2]. Virchow's case was possibly infected with the same organism. Kjellberg's case he regards as one of infection with *Isospora bigemina* ("*Coccidium bigeminum* Stiles, 1891"); and he regards Railliet and Lucet's two cases and the "coccidia" of Grunow as possibly coming under the same category. Blanchard's "*Eimeria hominis*" (Kunstler and Pitres's case) he considers a doubtful form, and he rejects the cases of Jürgens and Thomas.

The intestinal coccidia of man Braun regards, for the most part, as identical with those of the rabbit (*Eimeria perforans*); and he includes all these in the one species "*Coccidium hominis* Rivolta, 1878." As human cases he mentions those described by Eimer, and by Railliet and Lucet—the latter, as noted

[1] *Vide* Labbé (1896), p. 545; also his list of hosts and parasites, p. 562.

[2] I may note in passing that in the English translation of Braun's work (Braun, 1906, p. 79) there is an amusing mistranslation of a passage dealing with one of Leuckart's cases. We read: "Perls discovered coccidia in an old preparation of Sömmering's agglomerations"—a statement which recurs in the new version of Braun's *Animal Parasites*, in which the section on the Protozoa has been re-edited by H. B. Fantham. Presumably the translator mistook v. Sömmerring's collection ("Sammlung") of specimens for some kind of pathological accumulation ("Ansammlung") in the liver!

above, being possibly, however, *I. bigemina*. "Grassi's and Rivolta's cases" may, he thinks, have been infected with the same organism (*i.e. E. perforans*). Braun (1903, fig. 33, p. 67) gives, moreover, what purports to be a figure of the intestinal coccidia of man. It is labelled "*Coccidium hominis* (Riv.) in sporulation. (After Riek)." It is, however, in reality copied from the figures given by M. Rieck (1888), whose drawings depict *Eimeria perforans* from the rabbit[1].

Minchin (1903) does not discuss the coccidial parasites of man; but in his table of hosts and parasites—which is based upon that of Labbé (1899)—he enters "(?) *Coccidium cuniculi* (Riv.)" as occurring in the human liver, and "*C. perforans* var. (Kjellberg 1860)" and "*C. bigeminum*, Stiles" as inhabiting the human intestine. Later (Minchin, 1907) he merely remarks that "the coccidia...are alleged to occur in man, but no case of human coccidiosis has yet been satisfactorily investigated" (p. 67). In his latest work on the Protozoa (Minchin, 1912) the coccidia of man are—so far as I can discover—not even mentioned.

Lühe (1906), in his article in Mense's *Handbuch*, devotes some attention to the coccidia of man. He notices the cases of Gubler, Silcock, Dressler, Sattler, Perls, Eimer, Railliet and Lucet—not failing to add, of course, "the cases of Grassi and Rivolta"—and makes the sweeping statement that "in all these cases probably *Eimeria stiedae*[2] was present." It is almost incredible that anybody could thus incorporate the human parasites observed by Dressler, Eimer, and Railliet and Lucet, and the "bodies" found by Rivolta and Grassi, into one common species—the species occurring in the liver of the rabbit. Lühe's opinion is so obviously untenable, indeed, that further criticism would be superfluous. It only remains to add that he regarded Kjellberg's case as one of infection with *Isospora bigemina*, though somewhat questionable; and Blanchard's "*Eimeria hominis*" as a highly doubtful organism.

Bulloch (1907), in Allbutt and Rolleston's *System of Medicine*, in the main follows Leuckart and Blanchard. He regards the intestinal coccidia of man as *Eimeria perforans* (called *Coccidium hominis*) and the hepatic forms as *Eimeria stiedae* (called *Coccidium cuniculi*). Under the former he mentions Eimer's and Railliet and Lucet's cases—to which are added the inevitable "cases of Grassi and Rivolta"; and under the hepatic cases those of Gubler, Perls, Dressler, Sattler, and Silcock[3]. Virchow's (own) case is placed doubtfully in the same

[1] I mention this because Braun's figures of "*C. hominis*, after Riek" have already been copied as illustrations of the human parasite. They are very misleading, and their identification has given me some trouble. I may note here also that a figure of the "coccidium of man," called merely "*Coccidium hominis*" (without reference or acknowledgment), is given by Cammidge (1914), and appears to be a considerably retouched imprint from the same original.

[2] Lühe gives a list of synonyms of this species, in which he includes *Eimeria perforans*. I note that "*Coccidium perforans* Leuck." occurs in it twice, whilst *C. oviforme* Leuckart—perhaps the commonest of all—is omitted. Rivolta's (1878) genus *Psorospermium* is wrongly given as "*Sporospermium*."

[3] Concerning this case Bulloch adds that it was "an undoubted case of infection with *C. cuniculi*." That it was a case of coccidiosis may be readily admitted, but that the parasites were

group, but Kjellberg's case is omitted. Bulloch thus regarded all the human coccidia as identical with those of the rabbit[1].

Brumpt (1913), in his admirable *Précis*, also follows Leuckart and Blanchard. The hepatic coccidia of man are, he thinks, the same as those of the rabbit—*Eimeria stiedae* (called "*Coccidium cuniculi* (Rivolta, 1878)"). As a human case he quotes that of Gubler, and says "Dressler, Perls and Sattler, von Sommering (*sic*) and Leuckart, Silcock, etc., have recorded other cases." The intestinal coccidia of man he regards as *Eimeria perforans* (called "*Coccidium hominis* (Rivolta, 1878)"), citing Eimer's cases as an example; but he adds that *Isospora bigemina* "has twice been seen in man"—perhaps referring to the cases of Railliet and Lucet. Blanchard's "*Eimeria hominis*" is described as a "doubtful coccidium."

In the new edition of Kolle and Wassermann's *Handbuch* there is an article on Coccidiosis by Jollos (1913). He follows Lühe in regarding *Eimeria stiedae* and *E. perforans* as the same species, and in referring to it all human cases of infection—with the reservation that some may possibly have been infected with *I. bigemina*. He gives no detailed account of the human cases, and does not appear to have inquired into them for himself. Had he done so he could hardly have adopted Lühe's manifestly absurd conclusions.

Now it will be clear, I think, to anybody who considers the opinions of the various authors just enumerated, that—in spite of much difference in matters of nomenclature—there is a general consensus of opinion concerning the identity of the coccidial parasites of man. It is very generally agreed that they are not forms peculiar to man himself, but species which occur usually in other animals—man being regarded as a casual or accidental host. It is thus generally supposed that the hepatic coccidia of man are the same as those commonly found in the liver of the rabbit; and similarly that the intestinal forms found in man are the same as those occurring in the intestine of the rabbit, whilst probably including also those found in the cat and dog. This, as will be evident from what has been said in the preceding section, I take to be a very perverse view of the facts. It appears to me to be a quite unjustifiable tradition which originated at a time when our knowledge of the Coccidia was in its infancy, and which even then had but little real foundation.

But I will leave the discussion of this matter for the moment, and return to it again after considering the new facts, which more modern and competent investigation has brought to light, concerning the coccidia of man.

Eimeria stiedae is far from certain. One may well ask, indeed, whether anybody who saw the case at the time could have named the specific characters of *E. stiedae*: and from Silcock's description it is now impossible for anybody to determine the species of his organisms.

[1] With the exception of the doubtful organism of Kunstler and Pitres ("*Eimeria hominis* Blanchard"), which is also noticed.

C. The Coccidia described from Man in 1915 and later.

The year 1915 marks a definite advance in our knowledge of the coccidia of man, for towards its close two species were carefully studied and described for the first time by Wenyon. His observations will now be briefly reviewed.

Wenyon's cases. The organisms originally studied by Wenyon (1915, 1915 *a*) were found in soldiers invalided to England, suffering from dysentery and enteritis, from the Gallipoli campaign of 1915. In the faeces of some of these patients Wenyon discovered structures which were apparently the oocysts of a coccidium. These had previously been found in similar cases by Woodcock (1915) and Low (*vide* Wenyon, 1915), who, however, did not succeed in identifying them with certainty[1]. But Wenyon was able to observe all the stages of sporulation, and to demonstrate that the species is one which possesses an oocyst containing two tetrazoic spores. He therefore referred the parasite to the genus *Isospora*, though he did not give it any specific designation.

This coccidium has an oocyst resembling that of *Isospora bigemina*, but usually smaller, more pellucid and slender, and more delicate in appearance. It is a well-marked species, on the whole, and has since been found and recognized by a number of other observers (Woodcock and Penfold (1916), Roche (1917), Cragg (1917), etc.). A fuller description of the parasite will be given later, in the systematic part of this paper. For the moment it will suffice to note here that none of the later observers has been able to add anything of importance to Wenyon's description of the organism; and none of them has proposed a specific name for it[2] or assigned it to its proper systematic position. Although it is highly probable, from analogy, that the organism inhabits the epithelium lining the small intestine, this has not yet been actually demonstrated. Up to the present, although over sixty cases of infection have been recorded, the parasite is known only from the stages (oocysts) passed in the faeces. These are, nevertheless, sufficient to establish its systematic status with certainty.

In a later publication, Wenyon (1915 *b*) was able to record the discovery of a second coccidial parasite, found in the faeces of a single patient in the same series of cases. This organism was an *Eimeria*, with small spherical oocysts (about 20 μ in diameter), containing four oval dizoic spores and resembling *E. falciformis* of the mouse. As in the case of his *Isospora*, Wenyon was able to observe the characters most important for the systematic determination of the parasite, though he gave it no specific name.

[1] Woodcock (1915) found only the unsegmented, or partly segmented, oocysts: "for some reason or other no further development has ever been observed." He correctly concluded, however, though on very slender evidence, that "the coccidian is apparently of the *Isospora* type rather than the *Coccidium* type."

[2] The name *Coccidium isospora* employed by Savage and Young (1917) I take to be a misprint or *lapsus calami*. The organism is an *Isospora* and not a *Coccidium* (=*Eimeria*), and cannot be named in this fashion. If the authors' intention was to call the species *isospora*, then its name—for them—would be *Isospora isospora*.

This form also is well defined and easily recognizable: but it appears to be far less common than the *Isospora*, and, since its discovery, has apparently been found again in other cases by only one observer, Roche (1917). It will be dealt with in greater detail in the systematic section.

Since the publication of Wenyon's observations, I have found the oocysts of another human coccidial parasite in the faeces of a single individual. This organism, like the last-mentioned, is also an *Eimeria*—having a tetrasporic oocyst and dizoic spores. It differs considerably, however, from Wenyon's parasite; the oocysts being much larger (36 μ), and the spores long and pointed. I shall reserve a fuller description of it for the next section. I will merely note here that it is a well-defined and undoubtedly distinct form, specifically unlike any other *Eimeria* hitherto described.

As in the case of the *Isospora*, so with the two species of *Eimeria* found in man, it has not been possible as yet to determine the precise site occupied by the parasites in the tissues of their host. It appears highly probable, nevertheless, that they live in the epithelial cells of the mucous membrane of the small intestine. Wenyon's *Eimeria* is, at all events, so similar to the species infecting the mouse, that it would be expected to have a similar habitat in its host. It is to be remembered that the liver and the large bowel are—if we consider the Coccidia generally—unusual sites of infection in vertebrates.

Before I attempt to reconcile the observations made by Wenyon and later workers with the earlier researches and opinions of others, it will be well to recapitulate the facts recorded in the present section. At the same time I would emphasize a point of importance: namely, that our knowledge since 1915 contains, for the first time, some definite facts. It is no longer merely a mass of fragmentary findings connected by conjecture.

The conclusions which can be drawn from the most recent work are as follows: there are, in man, at least three different coccidia—one species of *Isospora* and two of *Eimeria*—whose oocysts are passed in the faeces of infected persons. All three are forms which, during their schizogonic and gametogonic stages, probably inhabit the epithelium of the small intestine. There is, at present, no reason to suppose that these species are not peculiar to man himself.

D. Discussion and Conclusions.

It is now possible, after our brief analysis of all the more important facts known about the coccidia of man, to attempt to reduce them to order: and I will therefore indicate how this can be done, and show how the more recently discovered facts can be reconciled with the conflicting statements and views which have hitherto been current.

It will be recalled that in an earlier section (Part I, A) we noted that the observations of the earlier workers indicated that there are at least two different coccidia parasitic in man—one probably an *Isospora*, inhabiting the intestine, the other an *Eimeria*, inhabiting the liver. In the last section (Part I,

C) we have just concluded that the facts more recently brought to light reveal the existence of at least three coccidia in man—one species of *Isospora* and two of *Eimeria*, all probably inhabiting the intestine. As regards the occurrence of a species of *Isospora* in the intestine, therefore, the records are in agreement: and we need have no hesitation, I think, in concluding that Wenyon's *Isospora* is probably identical with that originally discovered by Kjellberg (Virchow, 1860), and possibly the same as the parasite observed by Railliet and Lucet (1890).

When we attempt to go beyond this, however, the uncertainties increase. Were the coccidia found by Eimer (1870) of the same species? It is impossible to decide with certainty, because we have no description of them. But the nomenclature turns upon this question, because Eimer's coccidia were the first to receive a name—"*Cytospermium*" *hominis* (Rivolta, 1878). We must either retain this name, applying it to one of the forms now known, or else eliminate it altogether. When names are already in use, and can be applied without violence to known forms, it is, I submit, better to retain them than to replace them by new ones. In the present case, therefore, I propose, since I see no more acceptable solution of the problem, to identify Eimer's parasites with those of Kjellberg; and to suppose that both were the same organism (*Isospora*) whose oocysts were subsequently studied by Railliet and Lucet, and by Wenyon and others. This coccidium appears to be by far the commonest of those occurring in man: and if, therefore, Eimer, or anybody else, discovered a "coccidium" of which nothing is known but the fact that it inhabited the gut, then it is more likely to have been the *Isospora* than any other. If this solution be accepted—and for every reason I urge strongly that it should be—then the problems connected with the nomenclature of this organism are immediately simplified, and there is no necessity to introduce any new name. The parasite becomes *Isospora hominis* Rivolta, 1878, with a long list of synonyms, mostly due to incorrect determination of the species—a form which can always in the future be easily recognized. I shall adopt this view as that which involves the fewest difficulties and leads to least confusion.

It should be noted here that if Rivolta's name is rejected, on the grounds that the organisms (Eimer's) to which he gave it are not identifiable, then the next name which must be considered is that of Railliet and Lucet (1891). They found what they believed to be an *Isospora* in human faeces, and named it provisionally *I. bigemina* var. *hominis*. If we admit that the parasite was specifically distinct from *I. bigemina*—as there is every reason to believe—then its name could be arrived at by elevating the variety to specific rank. The coccidium would accordingly be known as *Isospora hominis* Railliet et Lucet, 1891. The chief difficulty involved in this procedure is, however, the same as that which we encountered in the case of Rivolta's name—the difficulty of determining precisely to what organisms the name was applied. For it is far from certain that the structures which Railliet and Lucet (1890) discovered really were the oocysts of an *Isospora*, or even of a coccidium. Their

description is far from clear, and in some ways even contradictory. Nevertheless, they may, I think, not unfairly be given the benefit of the doubt; and I shall therefore assume that their organisms were the same as those discovered by Kjellberg, seen later by Eimer, and rediscovered and first accurately described by Wenyon. It will be noted that even if we reject Rivolta's name, and accept that of Railliet and Lucet, the name of the organism itself will still be the same—*Isospora hominis*—differing only in the name of the authority cited for it. The fact that this name should have been twice introduced for parasites possibly identical with Wenyon's, and not certainly identifiable with any other organisms, supplies a sufficient reason, I think, for its retention in

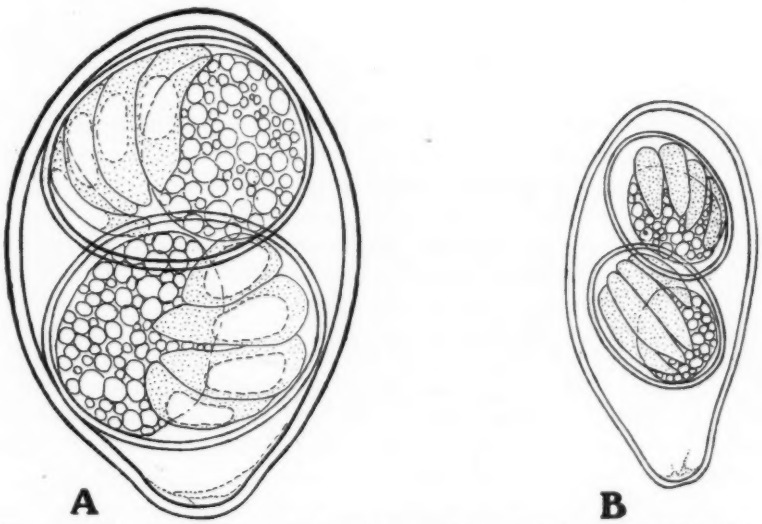

Fig. 1. Fully developed oocysts and spores of *Isospora bigemina* (A) from cat, and *I. hominis* (B) from man. Camera lucida drawings. Magnification approximately 2000 diameters.

this sense; whilst to abolish it could serve no useful purpose, and might even lead to greater confusion in the future.

We have already seen that many authors have asserted or conjectured that the *Isospora*, which they believed to occur in man, is the same as that occurring in cats and dogs[1]—namely, *Isospora bigemina*. For such a view there is not a vestige of evidence. In appearance the oocysts found in human faeces are quite unlike those from the faeces of the cat (cf. Text-fig. 1, A and B). The latter are usually much larger, though subject to great variation in size; their walls are thicker, and brownish in colour; and they are plumper and more egg-

[1] It may be pointed out that there is still no really conclusive evidence to prove that the *Isospora* of the cat is the same as that of the dog, or that both are merely varieties of one species. It is quite possible that they are distinct species. The matter requires further investigation.

like in their proportions. In comparison with them the oocysts of the human parasite are small, colourless, elongate, and generally more fragile in appearance. It would be almost impossible, I think, for anybody who had carefully compared the forms from the two different hosts to believe that they belong to one and the same species[1]. I have no hesitation, therefore, in concluding that the parasite of man is not *Isospora bigemina*, but belongs to a separate and well-marked species.

The *Isospora* of man can therefore be referred without much difficulty to its correct systematic position; and such problems as are raised by its nomenclature, and by the earlier records of human coccidiosis, are capable of fairly simple solution. But can the same be said about the species of *Eimeria* recorded from man? Are the new and old observations equally susceptible of reconciliation? The answer is, as I will now try to show, in the affirmative, in spite of the confusion which appears on the surface.

As we have already seen, the older workers appear to have discovered a species of *Eimeria* which inhabits the human liver. So far as can be ascertained from the fragmentary records, this organism resembles *Eimeria stiedae*, the species found in the liver of the rabbit. It is highly probable, however, that it is *not* this species, notwithstanding the fact that it has, by general consent, invariably been referred to it. No evidence for such a view has ever been brought forward, either experimental or morphological. There is not a particle of proof that the spores of the hepatic coccidia of the rabbit are capable of infecting man, or that those of the parasite of man can infect the rabbit. The allegations[2] which one constantly meets with in this connexion since the time of Leuckart are wholly without foundation, and there can be little doubt that they are merely text-book traditions of the type familiar to everybody[3]. Leuckart's original reasons for regarding the two forms as identical are too puerile to require further discussion. Moreover, his own work furnishes us with the only extant figures of the human parasite; and these—assuming that they are correct—supply the chief evidence, from a morphological standpoint, to prove that the hepatic coccidia of man and rabbit belong to distinct species

[1] Cf. Wenyon and O'Connor (1917): "The isospora of cats is very common in Alexandria, but the oocysts are quite unlike those of the isospora of man." I may add that *Isospora* is equally common in cats in London, where even quite young kittens are almost always infected. I have examined more than 100, and have rarely failed to find oocysts in their faeces—often in enormous numbers.

[2] *E.g.* "Green food-stuffs, contaminated with infected rabbit excrement, are infective to man" (Fantham, 1917).

[3] There is a similar tradition, equally devoid of foundation, concerning the "red diarrhoea," or dysentery, of cattle. Many authors assert that this is caused by *Eimeria perforans* (or *E. stiedae*) —the coccidium of the rabbit's small intestine (or liver). Even Doflein (1911) in the third edition of his text-book states this as a fact. Nevertheless, there is not a single fact to support such a view, and abundant evidence to refute it. The parasite of cattle, which lives in the large intestine, is morphologically quite unlike *E. perforans* (having small rounded oocysts, for example) and appears incapable of infecting rabbits. There can be no doubt that it is a distinct species (*E. zürnii* Rivolta, 1878) having no connexion whatever with the rabbit coccidia. Cf. Züblin (1908) and other recent works on this subject.

(see Text-fig. 2, p. 192). It appears highly probable, therefore, that the hepatic coccidium of man is not *Eimeria stiedae*, but a smaller and similar form which requires further investigation.

The two new species of *Eimeria* discovered in the faeces of man—that described by Wenyon (1915 *b*), and the form described in the present paper—are still less like *E. stiedae*. They cannot possibly belong to this species[1], and must be regarded as new. There is, indeed, a possibility that Wenyon's *Eimeria* is *E. falciformis*—the species which commonly occurs in the mouse. But there is no evidence to prove this, and it is, I think, preferable to regard the two forms as separate species. There are many good reasons for believing that most of the protozoal parasites of the human bowel are peculiar to man—not forms proper to other hosts, and accidentally acquired by him. Many facts point to this conclusion.

It seems clear, then, that there must be at least four distinct species of coccidia, belonging to two different genera, which are able to parasitize human beings. These are (1) *Isospora hominis*, already long known, but until the work of Wenyon, ill described; (2) a species of *Eimeria* discovered by Wenyon, resembling the form found in the mouse, but hitherto unnamed; (3) another species of *Eimeria*, to be described and named in the present paper; and (4) probably a third species of *Eimeria*, which, unlike the three intestinal parasites just enumerated, inhabits the liver. This last organism has been seldom seen, imperfectly studied, and incompletely described; so that it is not only unnamed but at present unnamable, because the characters upon which its specific determination depends are as yet unknown.

These conclusions appear to me to be fully justified; and I take them accordingly as a basis upon which to build a systematic account, which now follows, of the coccidia of man.

PART II.

SYSTEMATIC AND DESCRIPTIVE.

In this section I propose to give a brief systematic account of the four coccidia of man whose existence has been inferred from the facts analysed in the foregoing pages. I shall describe each species, as far as this is possible in the present limited state of knowledge, of all these parasites, and add such further details concerning their distribution, pathogenicity, etc., as can be gleaned from all available sources.

It has already been pointed out that one of the four parasites to be considered is a species of the genus *Isospora*, whilst the other three probably all belong to the genus *Eimeria*. It is necessary, however, to say something more about these two genera, as they offer certain difficulties to the systematist.

[1] Cf. figs. 2 and 3, Pl. VIII, and Text-fig. 2, B.

The genera **Eimeria** and **Isospora.**

Eimeria. This genus is one of the three original genera of coccidia founded by Aimé Schneider (1875). After passing through various vicissitudes, it can now be regarded as firmly established and well characterized. For present purposes, the most distinctive characters of the genus are those supplied by the oocysts and spores of the parasites. Each oocyst contains, when fully developed, four spores, each of which encloses two sporozoites. *Coccidium* Leuckart (1879) is a synonym of *Eimeria*, and is the name still preferred by some authors[1], though indefensible on grounds of priority. The type species of *Eimeria* is *E. falciformis* Eimer (1870)[2], the common parasite of the mouse. The researches of Schaudinn (1900) removed most of the difficulties connected with the genus, which was afterwards firmly re-established by Stiles (1902), and Lühe (1902). It will be unnecessary to enter into further details here.

Isospora. This genus is not so easily dealt with; in fact, it presents several difficulties and cannot even now be regarded as definitively established. The difficulties are due to our present ignorance regarding the type species, and they cannot be decisively removed until this has been reinvestigated.

The genus *Isospora* was founded by Aimé Schneider (1881) for an organism which, unfortunately, he did not study thoroughly, and of which he has left a very imperfect description. He found the parasite in "a little black slug"—he does not say in what organ—which he captured at La Fère (Aisne). He did not record its size. He proposed to call it *Isospora rara* n.g., n.sp.[3], but later referred to it himself as *Isospora incerta*[4]. He characterized the genus (and species) as follows: "psorosperm spherical, as in the preceding genus[5], with contents undergoing division into two sporoblasts, which develop into two regularly pear-shaped spores, containing pretty numerous sporozoites[6]." Schneider's figures are hardly more illuminating, though it seems probable from them that the spores each contained approximately four sporozoites—at all events, probably not more than four. It is thus possible to maintain that *Isospora* denotes, according to Schneider's own definition, a coccidian whose oocyst contains two polyzoic spores: or alternatively, that the oocyst really contained two tetrazoic spores. Subsequent authors have sometimes adopted the first interpretation, sometimes the second. Nobody, however, appears to have found the parasite again; and it offers certain difficulties to

[1] Minchin (1912) even goes so far as to say that the proposal to replace the name *Coccidium* by *Eimeria* is "contrary to public policy, and should not be followed, anything in the law of priority notwithstanding" (p. 346). I do not agree with this, and I do not believe that public policy is violated by obeying the law in this case. At all events the substitution of names referred to has already been effected by most writers, so that to revert to the name *Coccidium* now would not only contravene the law, but would also lead to still greater confusion.

[2] Called "*Gregarina*" *falciformis* by Eimer.

[3] Aimé Schneider (1881), p. 401.

[4] *Op. cit.*, description of Pl. XXII, figs. 65–72, p. 403.

[5] This refers to the genus *Klossia* Ai. Schn.

[6] "À corpuscules falciformes assez nombreux."

those who would look for it. For it is evidently uncommon, it occurs in an unknown organ of a host whose species can only be guessed[1], and it can only be known, when found, by its resemblance to Schneider's imperfect figures.

Labbé (1893), accepting *Isospora* as a form possessing two polyzoic spores, introduced the new generic name *Diplospora* for a coccidial parasite which he had found in birds, and which possessed two tetrazoic spores. In his subsequent works (Labbé, 1896 and 1899) he adhered to this nomenclature: but other workers have considered that *Diplospora* is a synonym of *Isospora*, believing *Isospora rara* to have been also a coccidium possessing two tetrazoic spores. This is the view generally taken, and it has the support of such authorities as Laveran and Mesnil (1902) and Schaudinn (1900). On the other hand Léger (1911), a great authority on the Coccidia, still prefers to regard *Isospora* and *Diplospora* as separate genera, believing that so accurate an observer as Schneider could not have mistaken a tetrazoic for a polyzoic spore. Minchin (1903, p. 232, footnote) has expressed the same opinion.

For my own part I am disposed to assume the standpoint of Laveran and Mesnil (1902) and to regard *Diplospora* Labbé as a synonym of *Isospora* Schneider; and I shall follow them, and the majority of recent authors, in referring the coccidia which produce oocysts containing two tetrazoic spores to the genus *Isospora*. The two best known species of this genus, though neither can be regarded as the type, are the forms generally called *I. bigemina*, from the intestines of cats and dogs, and *I. lieberkühni* from the kidney of the frog[2]. The human parasite which I shall refer to this genus—following Wenyon (1915)—bears the closest resemblance to *I. bigemina*, with which, as we have already seen, it has frequently been confounded.

The coccidial parasites of man can thus be referred to their genera, according to the characters furnished by their oocysts and spores, in the following way:

Genus 1. *Isospora* Aimé Schneider, 1881. (= *Diplospora* Labbé, 1893.) Oocyst containing two tetrazoic spores.

Genus 2. *Eimeria* Aimé Schneider, 1875. (= *Coccidium* Leuckart, 1879.) Oocyst containing four dizoic spores.

[1] It is sometimes stated that *Isospora rara* occurs in the kidney, and that its host is *Limax cinereo-niger* (cf. Doflein, 1911): but these are mere guesses—plausible, no doubt, but still quite unverified.

[2] I have already had occasion to note on a previous page that the correct name of "*Isospora bigemina* Stiles, 1891," is probably *I. rivoltae* Grassi, 1879. The parasite of the frog was first found by Lieberkühn in 1854 but not named by him. Labbé named it *Klossia lieberkühni* (in 1894) and later *Hyaloklossia lieberkühni* (1896, 1899). Laveran and Mesnil (1902) regarded *Hyaloklossia* as a synonym of *Isospora*, and therefore called the parasite *Isospora lieberkühni* Labbé, a name which it has since borne. It appears to have been overlooked, however, that the parasite was possibly first named by Rivolta (1878), who called it *Cytospermium ranae*. Its correct name therefore would be *Isospora ranae* Rivolta, 1878. I may also note here that it is possible to regard *Cytospermium* as the "correct" name of the genus here called *Isospora*, and to regard the parasite of the frog as its type. My own view is that it is far better to eliminate *Cytospermium* altogether, on the grounds that it was never defined, and included a heterogeneous assemblage of forms.

The species of human parasites belonging to these two genera will now be named and described as far as is possible.

(1) **Isospora hominis** Rivolta, 1878 (emend.).

Psorospermien (Kjellberg's), Virchow, 1860.
Psorospermien?, Leuckart, 1863.
Psorospermien, Eimer, 1870.
Cytospermium hominis Rivolta, 1878.
Coccidium perforans Leuckart, 1879.
Coccidien, Psorospermien, Bütschli, 1882.
Coccidium perforans (Leuckart) Blanchard, 1889.
Coccidies intestinales, Railliet et Lucet, 1890.
Coccidium bigeminum var. *hominis* Railliet et Lucet, 1891.
Coccidium bigeminum (Stiles) *p.p.*
Coccidium perforans (Leuckart) *p.p.* } Railliet, 1895.
Coccidium hominis (Rivolta) Labbé, 1896.
Coccidium hominis (Rivolta) *p.p.*
Coccidium bigeminum (Stiles) *p.p.* } Blanchard, 1896.
Coccidium perforans var. Kjellberg, Labbé, 1899.
Coccidium hominis (Rivolta) *p.p.*
Coccidium bigeminum (Stiles) *p.p.* } Blanchard, 1900.
Coccidium bigeminum (Stiles) Doflein, 1901.
Coccidium perforans var. (Kjellberg) *p.p.*
Coccidium bigeminum (Stiles) *p.p.* } Minchin, 1903.
Coccidium hominis (Rivolta) *p.p.*
Coccidium bigeminum (Stiles) *p.p.* } Braun, 1903.
Eimeria stiedae (Lindemann) *p.p.*
Isospora bigemina (Stiles)? *p.p.* } Lühe, 1906.
Coccidium hominis (Rivolta) Bulloch, 1907.
Isospora bigemina (Stiles) Doflein, 1911.
Coccidium hominis (Rivolta) *p.p.*
Coccidium bigeminum (Stiles) *p.p.* } Brumpt, 1913.
Eimeria stiedae (Lindemann) *p.p.*
Isospora bigemina (Stiles)? *p.p.* } Jollos, 1913.
Coccidia, ? *Isospora*, Woodcock, 1915.
Isospora, Wenyon, 1915.
Coccidium (*Isospora*) Wenyon, 1916.
Isospora, Woodcock et Penfold, 1916.
Isospora sp. Dobell, 1916.
Isospora sp. Dobell, 1917.
Isospora sp. Dobell et Stevenson, 1917.
Isospora, Roche, 1917.
Coccidium (*Isospora*) Wenyon et O'Connor, 1917.
Isospora, Cragg, 1917.

Isospora bigemina var. *hominis* (Raill. et Luc.) Fantham, 1917.
Coccidium isospora Savage et Young, 1917.
Coccidia (isospora), Martin, Kellaway et Williams, 1918.
Coccidium (*Isospora* sp.) Boney, Crossman, et Boulenger, 1918.

(See Pl. VIII, fig. 1, and Text-fig. 1, *B*, p. 177.)

The history of our knowledge of this parasite has already been dealt with in some detail. So far as I can judge it was originally discovered by Kjellberg, about 1860, in the villi of the small intestine. It was then seen—probably, but not certainly—though not named, by Eimer (1870); and first named, but not seen, by Rivolta (1878). Railliet and Lucet (1890) possibly found its oocysts in the faeces for the first time, but this is somewhat doubtful; and Rivolta and Grassi certainly did not find them, though often alleged to have done so. The first correct and clearly recognizable account of the oocysts and spores is that of Wenyon (1915, 1915 *a*). Drawings of these have been published by Wenyon (1915 *a*, 1915 *c*)—the latter work containing also some good photographs of the oocysts, taken by Dr A. C. Stevenson. Since the appearance of Wenyon's description over fifty cases of infection with this organism have been recorded.

The schizogonic cycle of development in the intestine has yet to be investigated[1]. It is doubtless closely similar to that of *I. bigemina* in the dog and cat. The organism is accurately known, up to the present, only from its oocysts in human faeces. They are elongate ovoid in form, the narrow end being usually somewhat drawn out into a sort of neck. They vary in length from about 25μ to 33μ; their width, at the widest part, being about half their length (ca. 12·5μ to 16μ). The relative dimensions of the oocysts are not constant, however, though they are much oftener long and slender than short and plump. (See Plate VIII, fig. 1, and Text-fig. 1, *B*.) The oocyst wall (oocyst proper) is clear, colourless, and porcellaneous in appearance. It consists of at least two—and probably more—layers, and is impervious to most fixing fluids. A micropyle is probably present at the narrower end of the oocyst, but it is inconspicuous.

The oocysts of *I. hominis*, like those of *I. bigemina*, are usually discharged from the body in an unsegmented condition (cf. fig. 1, Pl. VIII), though occasionally oocysts already containing two sporoblasts may be found in the freshly passed faeces. Development of the spores takes place outside the host, and requires several days for its completion. In this respect *Isospora hominis* differs conspicuously from the two species of *Eimeria* to be next described. At the time of its ejection from the body of the host, the oocyst generally has its protoplasmic contents contracted into an almost spherical mass, filled with brightly refractile granules. A clear area amidst these is often discernible, and probably represents the nucleus. (Cf. fig. 1, Pl. VIII.)

[1] The statements concerning the schizogony, fertilization, etc., of this form, made by Wenyon (1915 *a*), are presumably inferences from what is known of the development of allied forms.

Outside the human body, the protoplasm of the oocyst segments into two round sporoblasts which soon become ovoid and develop into spores, which are similar in structure to those of other species of the genus. The spore measures about 12 μ to 14 μ in length, 7 μ to 9 μ in breadth. The sporocyst is fairly thick, and contains, when the spore is mature, the four vermiform sporozoites characteristic of the genus, and a large granular sporocystic residue. (Cf. Text-fig. 1, *B*.) Very frequently one or two small and irregular bodies are to be seen at the narrower end of the oocyst. They are present before the segmentation of its contents (cf. fig. 1, Pl. VIII), and therefore do not represent a true oocystic residuum, which is absent in *I. hominis*. The sporozoites are long and narrow. Their internal structure is difficult to make out, but each probably possesses a nucleus situated subterminally.

Wenyon and O'Connor (1917) have recorded that an abnormal development may sometimes occur in which the oocyst forms "only a single sporocyst containing 8 sporozoites." I have seen a similar abnormality very rarely in *I. bigemina* from the cat.

Occurrence. The oocysts of this species were found by Wenyon (1915) in the faeces of patients invalided to England from Gallipoli, and suffering from dysentery and other intestinal ailments. They had previously been noticed in the same class of cases by Woodcock (1915) and Low (*vide* Wenyon, 1915). Wenyon found altogether fifteen infected cases among 556 examined (Wenyon, 1916); and one further case was subsequently found in completing the examinations of this whole series of 775 patients (Dobell and Stevenson, 1917). Woodcock (1915) and Woodcock and Penfold (1916) have recorded in all ten further infections found in the same class of cases[1], and I have recorded one (Dobell, 1916). All the infected persons had served in the Eastern Mediterranean War Area; and most, if not all, of them had been on the Gallipoli Peninsula. Somewhat later Roche (1917) found fifteen *Isospora* infections among 893 patients suffering from dysentery and diarrhoea at Salonika. Wenyon and O'Connor (1917) record a case found in Egypt, and note that "it seems probable that the infection came from Gallipoli." Savage and Young (1917) found six cases, out of 1088 men examined, among troops who had mostly served in Egypt, Gallipoli, Mesopotamia, and Salonika. Another case is recorded in Egypt by Martin, Kellaway, and Williams (1918), who examined 422 patients suffering from diarrhoea and dysentery. Cragg (1917) found four cases of infection among 613 patients—invalided for "dysentery and allied complaints" from Mesopotamia—whom he examined in Bombay: and Boney, Crossman, and Boulenger (1918) have quite recently stated that among 890 British patients with dysentery and other intestinal disorders,

[1] The two patients passing "coccidian cysts" recorded from Manchester by Williamson (*Journ. Roy. Army Med. Corps*, 1917, xxviii, p. 451), and the four patients in Liverpool infected with "Coccidia (apparently Isospora type)" mentioned by Fantham (*Lancet*, 10 June, 1916), probably belong to the same category. The patients were men from Gallipoli, but their infections were not accurately diagnosed. Cf. Dobell (1917).

examined at a base hospital in Mesopotamia, they found seven infected with *Isospora*. Finally, O'Connor[1] has found nine more infected cases in Egypt and Sinai among British troops and natives, of whom he examined some 3800 in 1916 and 1917.

Castellani (1917) states that "coccidiosis is, comparatively speaking, common in the Balkans," and mentions that he has seen "six cases, all in Macedonia." It is possible that these too were *Isospora* infections, but in the absence of any further information it is impossible to ascertain what organisms he studied.

Sangiorgi (1918) has quite recently described a case—a soldier suffering from nephritis—in which he believes he has found *Isospora bigemina*. The oocysts were found in the faeces, and bodies interpreted as schizogonic stages in the urine. It seems probable that this patient was really infected with *I. hominis*, though from the dimensions of the parasites—if they are correctly stated—this is doubtful. They can hardly have been *I. bigemina*, as the author believes. The "stages" found in the urine seem also open to question.

It will thus be seen that about seventy cases of infection with *Isospora hominis* have now been definitely recorded: and to this list several doubtful cases, both ancient and modern, should possibly be added. It is noteworthy that all the infections recorded since 1915 have been found in men who had been in Egypt, Gallipoli, Salonika, or Mesopotamia. The "dysentery" cases invalided to England from Gallipoli in 1915 showed a fairly high percentage of infection with *I. hominis*—2·7 per cent. in Wenyon's (1916) original series—but since then the infection has apparently disappeared in this country, even from among the same class of cases. At all events, I have not found the oocysts of *Isospora* in the faeces of any patients from Gallipoli or elsewhere since 1916, and inquiries which I have made of others have likewise failed to elicit a single case of infection in the patients invalided to this country during the last two years.

In most of the cases infected with *Isospora* the infection appears to have been small and transitory. An extreme instance is a case studied by Dr Stevenson and myself (1917), in which the stools were examined—sometimes most exhaustively—on six occasions, and only a single oocyst was ever found. Heavy infections appear to be uncommon, but have been found by Wenyon (1916), Roche (1917), Cragg (1917), and O'Connor. Roche (1917) states that "as a rule these parasites disappeared within a few days, but in one patient they were found in daily examinations for three weeks."

PATHOGENICITY. There is, up to the present, no proof that *I. hominis* is a pathogenic parasite. Since it is, like all other coccidia, an organism which lives at the expense of the tissues of its host, it is clear that it may be able to produce a pathological condition: nevertheless, even in the most heavily infected cases,

[1] Capt. F. W. O'Connor, R.A.M.C., has very kindly permitted me to record this and other information (contained in the present section, and attributed to him) from his still unpublished work.

no clinically recognizable "coccidiosis" due to this parasite has yet been described. It is true that the oocysts have generally been found in the faeces of patients suffering, or who had recently suffered, from dysentery or diarrhoea; but this is probably merely because the faeces of persons with no intestinal ailments are not usually examined. When symptoms of intestinal disorder have accompanied the infection, they have generally been attributable to other causes. Cragg (1917), for example, records two fatal cases in which "there was a very heavy infection" with *Isospora*. One died from "the rupture of an old dysenteric ulcer," and *Entamoeba histolytica* was found in the stools: the other succumbed to bacillary dysentery—Shiga's bacillus having been isolated before death. He concludes that in both these cases "there was other adequate cause for the patient's condition, and no indication of the pathogenicity of the Coccidium." *Post mortem* examinations were made of both these cases, but unfortunately nothing is recorded concerning the state of the patients' small intestines[1].

In this connexion the case which I found (1916) at Walton Hospital may be mentioned. The patient was not suffering from any intestinal ailment, though he had had diarrhoea on the Gallipoli Peninsula in the previous year. He was in hospital for varicocele, and was one of the series of seventy non-dysenteric patients whose stools I examined for protozoal infections. No infections with *Isospora* were discovered among the 130 patients with intestinal ailments examined at the same hospital.

Treatment. No method of treatment has yet been discovered which will get rid of an *Isospora* infection—or, for that matter, of any coccidial infection in any animal. Emetine appears to be the only drug which has hitherto been tried. Wenyon and O'Connor (1917) treated an infected patient with emetine hydrochloride—administered hypodermically and by the mouth simultaneously—and record that the oocysts disappeared from the stools during treatment and were not found again for a month following. But O'Connor has since treated two more cases with emetine, and found that the drug was without effect. Similar observations are recorded by Savage and Young (1917). Moreover, it is to be noted that the oocysts of this parasite have several times been first found in the stools after the patients had been treated with emetine: and it is not uncommon for them to disappear completely without any specific treatment.

Attempts to infect animals experimentally. Several attempts have been made to infect animals other than man with *I. hominis*, but without success[2].

[1] Cragg (1917), for some reason which is not apparent, seems to have supposed that *Isospora hominis* inhabits the liver. He mentions that "nothing unusual was found in the liver" in one case; and says of the other, "unfortunately the liver was not examined." So far as I am aware, an *Isospora* which inhabits the liver has not yet been discovered.

[2] Fantham (1917) has recently claimed to have infected kittens with *I. hominis*, and says he produced in their intestines a "condition resembling that seen in the human intestine examined post mortem." This, however, has never been described, so far as I am aware; and the statement can hardly be accepted without some concrete evidence to support it.

Wenyon and O'Connor (1917) fed kittens and a mouse on the ripe spores, but none of them became infected. Subsequently O'Connor made a very carefully controlled experiment with two young puppies, but he was unable to infect either. Attempts of this sort are not easily carried out, as both puppies and kittens are frequently found in nature already infected with a species of *Isospora* of their own. Up to the present, therefore, there is no evidence to show that *I. hominis* can parasitize any host but man.

(2) **Eimeria wenyoni** n. sp.

Eimeria (Coccidium) Wenyon, 1915 *b*.
Coccidium (*Eimeria*) Wenyon, 1916.
Eimeria sp. Dobell, 1917.
Eimeria sp. Dobell et Stevenson, 1917.
Eimeria Roche, 1917.

(See Plate VIII, fig. 2.)

I have named this species in honour of its discoverer, who has given us the only description which we possess of the parasite[1]. It is possible, however, that the coccidia found in man by Eimer (1870) were of this species; and if so, then this is the organism which Rivolta (1878) named *Cytospermium hominis*, and to which other names have since been given. We have previously decided to regard Eimer's coccidia as *Isospora* however, so that it will suffice merely to note this possibility here. It will be unnecessary to add a long list of conjectural synonyms.

This species is known only from its oocysts, which are similar to those of *E. falciformis*[2]. They are passed in the faeces in a fully developed condition, with the spores and sporozoites already differentiated. (Cf. fig. 2, Pl. VIII.)

The oocyst is approximately spherical, with a diameter of about 20 μ. Its outer surface is rough and rugose, its inner smooth and lined with a delicate membrane. The four spores which lie within it are oval, and measure about 10 μ by 7 μ. The external surface of the sporocyst is rough and irregular (probably from the presence of adherent remains of the epispore). There is no oocystic residual body. Each spore contains two typical sporozoites, lying with their blunter ends directed towards opposite poles, and one or two highly refractile masses (sporocystic residua).

Wenyon (1916) found this coccidium in small numbers in a sample of faeces from a single case out of 556 whose faeces he examined. The patient was among those invalided to England from Gallipoli (in 1915) suffering from "dysentery" or intestinal derangement, and was No. 242 in the series of cases studied at the London Hospital. In completing the examination of this series—775 cases in all—no other infections with this organism were discovered (Dobell and Stevenson, 1917): nor was its presence in the faeces recorded by any other

[1] The description of the organism here given is taken from Wenyon's account (1915 *b*).
[2] The oocysts of *E. falciformis* are, however, typically somewhat oval, and not spherical.

worker engaged in the study of the same class of cases in this country (cf. Dobell, 1917)[1].

Since the publication of Wenyon's original and only case, the parasites have been found again—so far as I have been able to ascertain with certainty—by only one observer. This was Roche (1917), who found three cases of infection among 893 military patients, all suffering from dysentery or diarrhoea, whom he examined at Salonika. The parasites were recorded merely as "*Eimeria*"; but Captain Roche, in answer to my inquiries, kindly informed me that they were, so far as he could judge, identical with the forms described by Wenyon.

There are thus only four cases of infection with this organism known up to the present; and when the very large number of persons examined is taken into account, it must therefore be regarded as extremely rare. Of its pathogenicity nothing is known, and methods of specific treatment for the infection are still untried. It is possible that a severe infection may give rise to an enteritis, since the habitat of the parasite is probably the mucous membrane of the small intestine: but if we exclude the highly doubtful cases of Eimer (1870) the organism has not yet been found in the tissues, and the fact that the recorded cases of infection were found among patients suffering from intestinal complaints is probably without significance. Our knowledge of the geographical distribution of the parasite is limited to the fact that hitherto it has only been recorded in persons from the Eastern Mediterranean region.

(3) **Eimeria oxyspora** n. sp.

(See Plate VIII, fig. 3.)

I propose this name for a coccidial parasite whose oocysts I have found in human faeces, and which has not been previously described. As in the case of *E. wenyoni* there is a remote possibility that this is the organism which was seen in the intestine by Eimer (1870); but there is nothing to support such a supposition. And of the other coccidia previously recorded there is none which can be regarded with any plausibility as belonging to this species.

Up to the present I have found this organism in the faeces of a single individual only. The patient is a young man who has been in South Africa, Ceylon, and India, and has suffered since 1912 from amoebic dysentery of a chronic relapsing type, which has hitherto proved extremely refractory to every kind of treatment. He has been for some time under the care of my friend Dr G. C. Low (now Temporary Major, I.M.S.), with whom I have studied his amoebic infection. A brief account of the case from this standpoint has already been published. (*Vide* Low (1918), Case 2, "B. W.," p. 164.) I have made an exhaustive examination of the stools of this patient (on over forty occasions in the course of the last year) but have found the coccidial parasite in them only twice, and then in extremely small numbers.

[1] The "*Eimeria* sp." noted by Dobell and Stevenson (1917) and Dobell (1917) both refer to Wenyon's original case.

The oocysts are passed in the faeces with their contents already completely differentiated. In this respect, therefore, they resemble those of *E. wenyoni* and differ from those of *I. hominis*. In almost every other way, however, they are strikingly different from those of *E. wenyoni*—and, in fact, from those of every other species of *Eimeria* with which I am acquainted. The oocyst (Plate VIII, fig. 3) is spherical, with a diameter of about 36μ. Its wall is composed of at least two distinct layers—an inner, fairly thick and uniform, and an outer which appears composite, and is incrusted with adherent bacteria and other foreign particles from the faeces. The wall as a whole is faintly yellow in colour, but is quite transparent, so that the entire contents of the oocyst can be very clearly seen.

The oocyst contains, in addition to the four dizoic spores characteristic of the genus, a small oocystic residue composed chiefly of very brightly refractile granules, which may be more or less dispersed. The spores themselves are long and sharply pointed at both ends, their shape being like that of a whetstone. Their length is 30μ-32μ, and their width in the middle about 7·5μ. The sporocyst is composed, as usual, of a tough and uniform inner coat (endospore) and a deciduous thin outer membrane (epispore). The remains of the latter give the external surface of the spore a slightly uneven or frilled appearance, noticeable chiefly towards the extremities.

The two long and slender sporozoites lie within the spore, which they almost fill, in the typical manner, with their anterior ends[1] directed towards opposite poles. The anterior end of each is pointed, and wrapped round the posterior end of its fellow. The posterior end is rounded, and contains an oval body, lying subterminally, which is probably the nucleus. Between the latter and the posterior extremity there are always present two or three very bright and small bodies[2], fusiform in outline and longitudinally disposed. (Cf. fig. 3, Pl. VIII.) A few small and rather feebly refractile granules lie anterior to the nucleus and extending for a short distance forwards along the body of the sporozoite. There is a relatively small sporocystic residue, represented by a few bright and scattered granules lying near the middle of the spore.

The foregoing account is based upon a careful study of the living oocyst, as I have not been able to obtain any stained preparations. All the structures described are, however, easily made out in the living organisms; and they are shown, as accurately as possible, in fig. 3, Pl. VIII. Dr A. C. Stevenson, of the Wellcome Bureau, kindly photographed the oocyst from which this drawing was made. Unfortunately, however, the object is a very unsuitable one to photograph, as the spores all lie in different planes, and consequently cannot all be shown clearly at the same time. The photograph, though forming a

[1] I use these terms—anterior and posterior—for the ends which appear to correspond to the fore and hind extremities of other coccidial sporozoites which I have been able to study in greater detail—especially those of *Aggregata* and *Adelea*.

[2] I do not remember to have observed similar structures in the sporozoites of any other coccidium. I do not know what they are. They have a very bright and almost crystalline appearance.

permanent record, was therefore thought not so suitable for reproduction here as a carefully executed drawing.

I can add nothing regarding the pathogenicity of *E. oxyspora*. The patient in whose faeces the parasites were found has a very heavy infection with *Entamoeba histolytica*, and a small infection with *Ankylostoma*. The *Eimeria* infection was so small that, even if it had caused any clinical symptoms, it would hardly have been possible to distinguish these from the effects produced by the accompanying parasites. At present there is no indication that *E. oxyspora* is pathogenic; though since it is, like all other coccidia, a tissue-parasite, it is doubtless capable of doing damage to its human host. From analogy with other forms, one would expect that the habitat of the parasite is the epithelium of the small bowel; and therefore that the effects, if any, of its presence would be manifested as an enteritis.

It is perhaps worthy of mention that, as in the case of *Isospora*, it is probable that the administration of emetine has no curative effect upon an infection with this organism: for the case infected had already received large quantities of emetine, both hypodermically and *per os*, before the parasites were found in his faeces.

(4) **Eimeria** (?) sp., the hepatic coccidium of man.

"Cellules ovoïdes, ? œufs d'helminthes," Gubler, 1858.
"Corps oviformes," Davaine, 1860.
"Psorospermien," Leuckart, 1863.
"Psorospermi," Rivolta, 1873.
Coccidium oviforme Leuckart, 1879.
"Coccidien," "Leberpsorospermien," Bütschli, 1882.
Coccidium oviforme? (Leuckart) Balbiani, 1884.
Coccidium oviforme (Leuckart) Blanchard, 1889.
Coccidium oviforme (Leuckart) Silcock, 1890.
Coccidium oviforme (Leuckart) Railliet, 1895.
Coccidium cuniculi (Rivolta) Blanchard, 1896.
Coccidium cuniculi? (Rivolta) Labbé, 1899.
Coccidium cuniculi (Rivolta) Blanchard, 1900.
Coccidium cuniculi (Rivolta) Doflein, 1901.
Coccidium cuniculi (Rivolta) Braun, 1903.
Coccidium cuniculi? (Rivolta) Minchin, 1903.
Eimeria stiedae (Lindemann) Lühe, 1906.
Coccidium cuniculi (Rivolta) Bulloch, 1907.
Eimeria stiedae (Lindemann) Doflein, 1911.
Coccidium cuniculi (Rivolta) Brumpt, 1913.
Eimeria stiedae (Lindemann) Jollos, 1913.

(See Text-fig. 2, *A*.)

This species is added here chiefly for the purpose of directing attention to our ignorance of it. Hitherto it has been wrongly regarded as identical with the species which occurs in the liver of the rabbit (*Eimeria stiedae*).

Altogether there are but five recorded cases of infection with this organism; namely, the cases of Gubler[1] (1858), Dressler (Leuckart, 1863, 1879), Perls and Sattler (Leuckart, 1879), Perls and von Sömmerring[2] (Leuckart, 1879), and Silcock (1890). These were recorded respectively in Paris, Prague, Vienna, Giessen, and London. Gubler and Silcock have given some account of their patients: of the others practically nothing is known. Gubler has described the parasites—in the language of his day, and in ignorance of their nature—and Davaine (1860), Silcock, and Leuckart have noted their resemblance to *E. stiedae*. Dressler's rude sketches of the oocysts which he found have been reproduced by Leuckart (1863, 1879). Beyond these fragments nothing of any value can be gleaned concerning the coccidia themselves.

That the hepatic coccidium of man displays a general resemblance to *E. stiedae* seems probable from the testimony of Gubler, Davaine, Leuckart, and Silcock. We learn from the first that the oocyst is ovoid. This is confirmed by Dressler's drawings and Silcock's description, and is implicit in Leuckart's account. Gubler further noted the presence of a micropyle at one end of the oocyst. Nobody, however, has recorded the size of the latter; but from Dressler's drawings—the magnification of which is given by Leuckart—it is evident that its length is about 20 μ. It thus appears that the oocysts of the human hepatic coccidium are much smaller than those of *Eimeria stiedae*; and this is confirmed by Davaine (1860), who expressly states that they resemble the *smaller* forms found in the rabbit's liver[3]. Silcock alone seems to have seen the formation of spores within the oocyst. The other observers saw only the oocysts with unsegmented contents. But Silcock gives no description of the sporulation, and does not state how many spores and sporozoites were formed. He appears, however, to have believed that the human coccidium is similar, in these respects, to *Eimeria stiedae* as described and figured by Leuckart. Perhaps it may be concluded from this that the human parasite is also an *Eimeria*. Beyond this it would be rash to draw any conclusions as to its systematic status.

Dressler's drawings, as reproduced by Leuckart, apparently show four oocysts seen under a low magnification (330 diameters), in which little can be made out; and two larger (magnified 1000 diameters) which are apparently

[1] Gubler was the original discoverer of the organism.

[2] This case, as already noted, is very doubtful; nor is there much to support the one preceding it. They are cited here on the authority of Leuckart—not a very convincing authority where the Protozoa are concerned.

[3] The oocysts of *E. stiedae* are commonly 35 μ–37 μ in length. I have never seen any measuring less than 30 μ. It is noteworthy that Leuckart himself gives figures of *E. stiedae*, magnified 550 diameters (1879, fig. 106, p. 256), which are approximately equal in size to those which he gives of the human parasite (Dressler's figure, Fig. 114, *b* and *c*, p. 281) stated to be enlarged 1000 diameters.

oocysts with unsegmented contents—one of them degenerate and breaking up, the other with its contents contracted from the cyst. I have copied this last figure (Text-fig. 2, *A*), and give beside it (Text-fig. 2, *B*), an outline of a typical oocyst of *Eimeria stiedae* at a similar stage and the same magnification. Even when every allowance is made for differences of technique in draughtsmanship, it is difficult to believe that the objects from which these drawings were made were identical, or even strikingly alike. To me it seems certain that if Dressler's drawings were even only approximately correct, then the hepatic coccidium of man cannot be *Eimeria stiedae*. How it comes about that all authors, for some forty years, have unanimously declared the two organisms to be identical, is a mystery which need not be discussed further here[1].

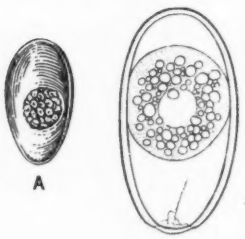

Fig. 2. A, oocyst, with unsegmented contents, of human hepatic coccidium. (From Leuckart, after Dressler.) B, oocyst of *Eimeria stiedae*, from gall bladder of rabbit: stage in development similar to A. (Drawn with camera lucida.) Both magnified approximately 1000 diameters.

From the statements of Gubler, Leuckart, and Silcock, it seems justifiable to conclude that the lesions produced by the human parasite resemble those seen in the rabbit, and caused by *E. stiedae*. Silcock's account, if correct, appears to indicate that the human parasite may also invade the spleen[2], and possibly the gut. The clinical picture of human hepatic coccidiosis—as presented by Gubler and Silcock—shows no particularly striking features. The condition is manifested, as might be expected, by enlargement of the liver, fever, and digestive derangements. It may be noted that the condition of Gubler's case was diagnosed before death as due to a hydatid cyst; whilst Virchow (1860), from Gubler's description, regarded it as probably a case of cancer, and Giles (1890) notes that it "reads suspiciously like one of ankylostomiasis."

From the information available I conclude that there is probably a coccidial parasite which occurs very rarely in the human liver; that it resembles *Eimeria stiedae*, but is considerably smaller, and probably belongs to the same genus, but to a distinct species; and that there is no evidence that *Eimeria stiedae* has ever been found in the human liver, or that this species can infect man[3]. It is clear that further observations alone can solve the problems connected with the human parasite, and the naming

[1] The explanation is doubtless that given by Bacon, in another connexion, some three hundred years ago. "For as things now are, if an untruth in nature be once on foot, what by reason of the neglect of examination, and countenance of antiquity, and what by reason of the use of the opinion in similitudes and ornaments of speech, it is never called down." *Adv. Learn.*, II, 1 (3).

[2] So far as I am aware, *E. stiedae* has never been found in this situation in the rabbit.

[3] It has been stated by Fantham (1917) that cases of infection with *Eimeria stiedae* have recently occurred "among patients from the Eastern war zones." I can find no authentic records to justify this statement, and the figure of "*Eimeria stiedae*" which accompanies it will hardly serve to carry conviction to those familiar with this organism.

of it may therefore be left to some future investigator who is fortunate enough to find it once more[1].

SUMMARY AND GENERAL CONCLUSIONS.

From a study of the organisms themselves, so far as this has been possible, and from an analysis of all accessible records relating to them, I conclude that there are four distinct species of coccidia which may parasitize man. These are: (1) *Isospora hominis* Rivolta, 1878 (emend.), discovered by Kjellberg in 1860, and recently investigated by Wenyon; (2) *Eimeria wenyoni* n.sp., a form discovered in 1915 by Wenyon; (3) *Eimeria oxyspora* n.sp., another new form, here described for the first time; (4) an undetermined species of *Eimeria* (?) which was discovered by Gubler in 1858. This last inhabits the human liver, whilst the three others probably live in the small intestine. Probably some seventy cases of infection with the *Isospora* have now been seen, mostly in persons who have been in the Near East; but the other parasites appear to be extremely rare, and little is known concerning their probable geographical distribution.

All these parasites are probably peculiar to man. There is no evidence that any of them is or can be parasitic in any other host. The prevailing belief that the coccidia of man are identical with those of rabbits, cats, or dogs, is therefore unfounded. Furthermore, there is as yet no good evidence to show that man may harbour any species of coccidia other than the four just enumerated. All these forms, however, require fuller investigation. They are here briefly and incompletely described from the data at present available.

There is at present no proof that the coccidia of man—with the probable exception of the species occurring in the liver—can produce a clinically recognizable pathological condition of "coccidiosis": and as yet no method of treatment which will eradicate an infection with any species has been discovered.

LONDON, *August* 1918.

[1] As it seemed possible that specimens or preparations from the case studied by the late Dr Silcock might still be in existence at St Mary's Hospital, my friend Capt. S. R. Douglas, I.M.S. (ret.), very kindly instituted inquiries on my behalf, and searched through the records and material in the Pathological Department. His efforts unfortunately were unavailing, but I take this opportunity of thanking him again for his help in the matter. Up to the present, therefore, I have had no opportunity of studying the parasites themselves from any case of human hepatic coccidiosis.

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DESCRIPTION OF PLATE VIII.

The figures in this Plate show the oocysts of the three human intestinal coccidia described in the text. They were all drawn originally at a uniform magnification (approximately 2000 diameters), and have been reduced to the size here shown (1600 diameters) in the process of reproduction. Figs. 1 and 3 were drawn, under a 2 mm. apochromat (N.A. = 1·40), from living oocysts. Fig. 2 is copied, with very slight modification, from Wenyon (1915 *b*). The oocysts are shown as they usually appear in freshly passed human faeces—those of the two species of *Eimeria* containing fully formed spores, that of the *Isospora* with its contents still unsegmented. (The fully developed oocyst of this species, containing two tetrazoic spores, is shown in Text-fig. 1, B, p. 177.)

Fig. 1. *Isospora hominis* Rivolta (emend.). Undeveloped oocyst.

Fig. 2. *Eimeria wenyoni* n.sp. Fully developed oocyst and spores.

Fig. 3. *Eimeria oxyspora* n.sp. Fully developed oocyst and spores.

A SHORT LABORATORY STUDY OF *SPIROCHAETA ICTEROHAEMORRHAGIAE*.

By Deputy Surgeon General P. W. BASSETT-SMITH, C.B., C.M.G., R.N., F.R.C.S., F.R.C.P.

(With Plate IX and 1 Chart.)

The occurrence of cases of spirochaetal jaundice from time to time in our over-seas forces, and the general interest of spirochaetal affections at the present time, both in man and animals, are my excuse for offering the following notes.

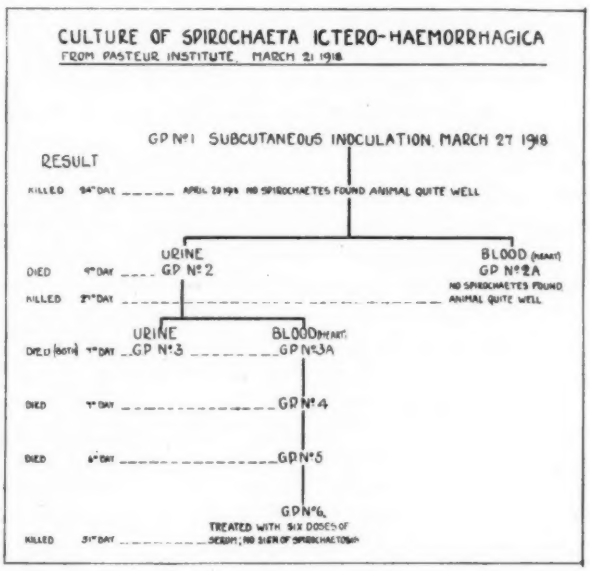
On the 21st March, this year, while in Paris and discussing the etiology of trench fever, Dr Pettit was kind enough to show me animals infected with the icterohaemorrhagica spirochaete and the cultured organisms under dark-ground illumination. He also gave me a living culture of the spirochaete in rabbit serum medium and some anti-serum prepared at the Pasteur Institute.

On arrival at the Royal Naval College, Greenwich, six days afterwards a guinea-pig (No. 1) was inoculated subcutaneously from the culture which had been kept at room temperature, the culture then showed abundant active forms of the "Leptospira" type (Nogouchi), also some contaminating cocci. On the 7th day a few spirochaetes were found in the animal's urine and the conjunctivae were slightly tinged. There were no spirochaetes in the blood but the red cells showed marked basophilic stippling. The animal then began to improve, no spirochaetes being again found in the blood and urine, and on the 24th day as it appeared quite well it was killed. The organs were normal and no evidence of jaundice was present. Blood and urine examined by dark-ground illumination and Fontana methods, and the organs by Levaditi all gave negative results for spirochaetes, and cultures into rabbit serum were also negative. Guinea-pigs (No. 2), however, were inoculated subcutaneously, one with fresh heart-blood and one with urine. The former showed no signs of infection and was killed on the 27th day and its serum preserved. The latter on the 9th morning appeared very ill with jaundice and irregular breathing; it died on the same day. *Post Mortem.* Subcutaneous tissues were moist, yellow, and haemorrhagic, kidneys intensely congested, spleen very friable, liver pale, and embryos in uterus dead. The heart-blood showed abundant spirochaetes.

Guinea-pigs (No. 3) were inoculated with heart-blood and urine; *both* of these developed the disease and died on the 7th day. Post mortem condition,

that of acute icterohaemorrhagica. No. 4 guinea-pig, inoculated with heart-blood; this died on the 7th day showing the usual acute symptoms with abundant spirochaetes in the blood and in the haemorrhagic urine. The heart-blood was inoculated into a fifth guinea-pig. On the 4th and 5th day about 1 c.c. of serum from guinea-pig No. 2*a* was given, but this either contained no immune body or insufficient, as the animal died on the 6th day. A sixth guinea-pig was inoculated on the 21st May from the heart-blood of No. 5, containing spirochaetes; on the 23rd, 24th, 25th, 27th, 29th, and 4th June, 1 c.c. of the Pasteur Institute curative serum was given subcutaneously. The animal never showed any signs of the disease, and was killed on the 51st day. At the autopsy all the organisms were healthy and no spirochaetes could be found, also its blood and urine were not infected by inoculation.

A white rat inoculated with infective blood was immune.



The experiment shows:

(1) The primary culture, though living when used for inoculation at Greenwich, had lost much of its toxicity, and though the animal when killed on the 24th day gave no microscopical or cultural evidence of spirochaetes, yet the urine by inoculation was lethal in nine days, giving rise to typical symptoms. The local infection had remained but the septicaemia had passed off, for the serum of an animal inoculated with the blood contained no protective antibodies.

(2) By passage the toxicity was gradually increased, killing on the fifth passage on the sixth day.

(3) An animal injected with this virulent blood was by repeated inoculations of the Pasteur Institute immune serum protected absolutely, as has been shown by Martin, Pettit, and Vaudremer.

(4) Blood and urine may be infective to animals, though careful examination of the fluids by staining, dark-ground illumination, etc., may fail to show any spirochaetes (at least this was so in my hands); this may occur in other spirochaetal disease, possibly trench fever.

(5) A white rat was found to be immune.

We may conclude that the spirochaete of the type culture is the cause of the disease and that perfect protection in animals may be obtained against a very virulent infection by the use of the anti-icterohaemorrhagic serum prepared in the Pasteur Institute; a protection found by Fiessinger, Lervy, and others, which extended to human cases. (The serum has therefore been supplied to Naval units in Flanders.)

The morphological characters of the organism have been fully described by Pettit and others, but under dark-ground illumination the long cultural form with its many minute turns and vesicular like body at one end are very remarkable, and the frequency with which these are interlaced together. Transverse fission appears very definite.

The smears from the organs show generally abundant forms; these are much shorter and have fewer turns. At death the kidneys are seen to be greatly enlarged, dark, congested, and haemorrhagic. On section the malpighian capsules are frequently blocked with red blood cells, a glomerular nephritis. The tubules were blocked with red cells and all the blood vessels were distended. The spirochaete is abundantly present in the organ at this time. The livers were generally pale, and on section showed areas of degeneration of whole lobules, with small cell infiltration round the portal vessels.

Finally I have to thank Prof. Louis Martin and Dr A. Pettit of the Pasteur Institute for providing me with the culture and immune serum, and Mr S. Mangham, M.A., for the micro-photographs reproduced in Plate IX.

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L. Martin and A. Pettit. *Bull. Soc. Med. Hôp.*, Paris, 1917, p. 1156.

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THE BIOLOGY OF *PEDICULUS HUMANUS*.

SUPPLEMENTARY NOTES.

(1) Colour and Light Reactions. (2) The influence on lice of temperature conditions in clothing and the absorption of radiant heat by cloth. (3) The influence of black, white, and coloured backgrounds and of coloured screens upon pigmentation in lice, proving that pigmentation is not hereditarily transmitted. (4) The relative proportions of the sexes.

By GEORGE H. F. NUTTALL, F.R.S.

(*From the Quick Laboratory, University of Cambridge.*)

(With Plate X and 1 Text-figure.)

1. COLOUR AND LIGHT REACTIONS.

Only one author has hitherto touched upon the subject of the reactions of lice towards colour, namely, Galli-Valerio (xii. 1916, p. 35), who, learning from a Serbian doctor that black clothing is stated to repel lice, carried out one experiment wherein he placed the insects upon variously coloured paper and counted the number that wandered to each colour. The results recorded permit of no conclusions, no particulars are given regarding the manner in which the experiment was performed, and consequently it remains to be determined if lice react to colour.

Sources of experimental error.

In *Parasitology*, x. pp. 176, 100, reference was made to certain reactions to light that are exhibited by *P. humanus* and how they wander away from a source of light toward the shade. Indeed, I have found that they are so sensitive to different intensities of illumination that if light from a window falls upon them from one side, whilst it is reflected in upon them from the opposite direction by a mirror, the insects wander away quickly from the window side to the mirror side of the experimental cell in which they are confined although the difference in the intensity of the illumination from the two sides is but slightly appreciable to the human eye. It is therefore essential in testing the preference of lice for white, black or coloured backgrounds that the insects should be exposed to *vertical* illumination. Slightly oblique rays will invariably cause them to wander to one side; this being conveniently observed by placing the cell upon a stool with a top that is rotated periodically.

Since experiments conducted with single or but few insects would consume much time, it is best to use many lice, but their tendency to collect in a mass

and to remain clinging to each other requires to be counteracted, otherwise it may vitiate the experiment. The difficulty is met through separating the insects prior to each test and distributing them singly over the floor of the experimental cell.

Methods.

My experiments were conducted on bright sunny days in May, and mostly in the open air on the flat roof of a building. The apparatus employed (Fig. 1) was of a simple improvised character. The lice were confined beneath an

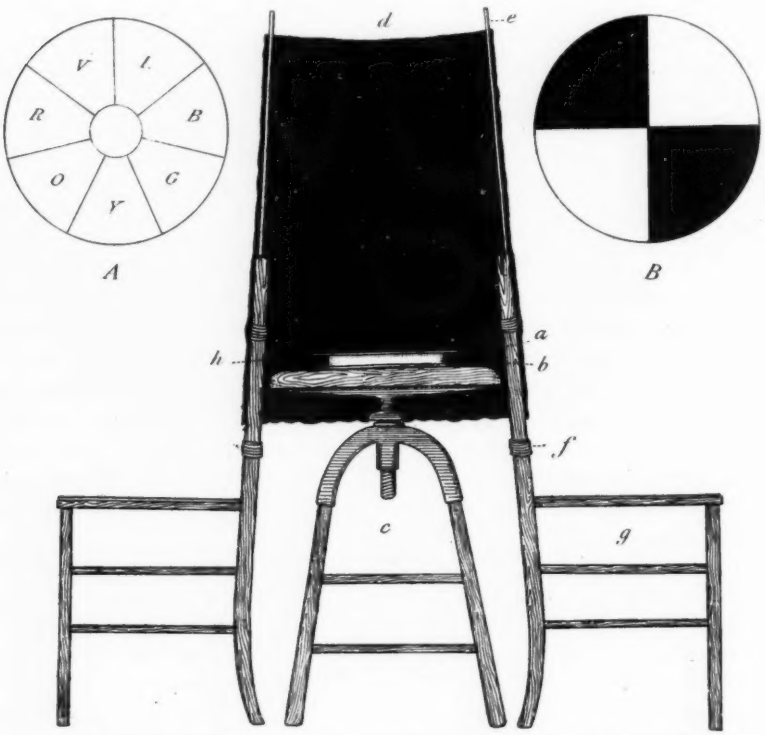

Fig. 1. Apparatus used for experiments on the reactions of lice to light and colour.

inverted, flat, circular glass dish, 22 cm. in diameter, with vertical sides 2 cm. in depth, hereinafter called the cell (Fig. 1 *a*). The cell rested upon a flat-topped stool (*c*) with rotating top. To prevent the light from falling otherwise than vertically upon the insects, the top of the stool was surrounded by a quadrangular funnel of dull black sateen (*d*), the funnel measuring ca. 60 cm. in height and 35 cm. across. The funnel was constructed by tying 4 rods of glass or bamboo (*e*) vertically by means of string (*f*) to the sides of the backs of two chairs (*g*) which were placed back to back on either side of the stool;

the sateen was wound smoothly around the rods and fixed in place with pins. The apparatus was placed in the shade of a low wall so that only bright diffuse light fell vertically into the funnel from the sky.

1. *Light and Shade.* To determine if lice seek the shade under vertical illumination, the cell was carpetted white, and half of it shaded by a black cover.

2. *Black and White.* To determine if lice prefer a black to a white background, a circular piece of white linen, with two quarters blacked with India ink (Fig. 1 *B*) served as a carpet for the cell.

3. *Colours.* Two methods were employed in testing the influence of colour[1]: (*a*) the cell was roofed by a transparent polychrome gelatin screen, being carpetted white, or (*b*) white light entered the cell from above, the carpet being polychrome. In both cases what appeared to my eye to be the colours of the spectrum were employed, the coloured areas being arranged radially (as shown in Fig. 1 *A*), a circular uncoloured surface, 5 cm. in diameter, being left in the centre. The dyes used for preparing the polychrome screen and carpet were the following:

	Dye	Wave lengths transmitted through the screen[2]
VIOLET	methyl violet	blue to w.l. 4900, and red beyond 6300
INDIGO	toluidine blue	indigo and blue to w.l. 4850
BLUE	methylene blue	indigo, blue and green to w.l. 5500
GREEN	lichtgrün	blue and green from w.l. 4600 to 5750
YELLOW	picric acid	whole spectrum except blue (down to 4700)
ORANGE	orange G.	green, yellow, orange and red below 5300
RED	trypanred	yellow, orange and red below Na line

The colour screen: seven tubes of ordinary 10 per cent. neutral nutrient gelatin, as used for bacteriological purposes, were warmed to melting point in a water-bath, and the requisite amount of each dye was added to each tube. A clean glass plate with lines of demarcation drawn thereon in melted paraffin, was placed on a levelling tripod, and each portion of coloured gelatin was poured over its apportioned area and spread with a glass rod. The paraffin lines set boundaries to the flow of the melted gelatin. After the gelatin had cooled and set, the glass plate was reversed over a shallow circular dish containing calcium chloride, whereby the gelatin became completely dried and presented the appearance of brilliantly stained glass.

The coloured carpet: corresponding lines to those described above were drawn on a piece of linen by means of a brush charged with melted paraffin.

[1] An attempt was made to use the spectrum from an arc light in a dark room, 25 lice being confined in a glass cell measuring 3 × 2 inches the floor of which was covered with the colours projected by the prism. The insects showed a definite tendency to wander to the walls of the box at the red and violet ends, which, however, may have been due to the obliquity of the rays and the insects seeking darkness. Mr Mills (*f. infra*) very kindly placed the apparatus at my disposal.

[2] I am much indebted to Mr W. H. Mills, M.A., for determining these wave lengths for me from photographs of the spectra kindly taken by Mr Frederick Stoakley at the Chemical Laboratory, Cambridge.

The linen was laid on a glass plate, and the various dyes were applied uniformly thereto by means of a feather upon the desired areas within the paraffined boundaries. The fabric became saturated with the dye, whilst the paraffin set limits to its spread. The surplus dye was absorbed with filter paper and the carpet was allowed to dry.

Experiments.

Light and Shade. Unfed and fed lice, in lots of 50, placed on a white paper carpet and illuminated vertically by a bright light, scattered uniformly on the floor of the cell, but after a time they gathered along the cell wall seeking to escape. When half of the cell was covered with black cloth, they sought the shade, only a few wandering about on the lighted surface.

White and black carpet. Unfed lice, in lots of 50, were placed on the black and white quartered carpet (Fig. 1 *B*) being illuminated from above. 80 per cent. were counted on the black and 20 per cent. on the white. When the apparatus was placed in a room and the light from the sky was reflected down vertically by a mirror, 81 per cent. were counted on the black and 19 per cent. on the white. The total counts in both series of observations were 491 black and 119 white.

Coloured screen. Unfed and fed lice in lots of 50, placed on a white paper carpet and illuminated vertically through the screen, gave the following counts after 15–20 minutes exposure:

	Set I. Lice unfed 16 hrs		Set II. Lice unfed 22 hrs		Set III. Lice fed 2–3 hrs before	
Colour	No. counted	%	No. counted	%	No. counted	%
Violet	104	52	33	22	82	41
Indigo	33	16·5	26	17·3	28	24
Blue	17	8·5	23	15·3	39	19·5
Green	13	6·5	14	9·3	17	8·5
Yellow	13	6·5	21	14	7	3·5
Orange	12	6	13	8·6	11	5·5
Red	5	2·5	20	13·3	16	8
In white centre	3		—		—	
Totals	200		150		200	

Coloured carpet. Two series of experiments were carried out, but they afforded no evidence that the lice were influenced by the colours on the carpet.

Conclusions.

The foregoing experiments demonstrate that lice, when illuminated by rays of light falling vertically upon them, seek the shade, and a black surface in preference to a white one. The experiments were conducted at 17–20° and 20–23° C.

When exposed upon white paper beneath a screen with bright spectral colours, either after fasting 16 hours (Set I) or 2–3 hours after feeding (Set III), the majority (52 per cent. and 41 per cent.) gathered beneath the violet, whilst

indigo and blue gave the next highest figures. In Set I the numbers fell steadily from violet down to red, in Set III the fall was less regular. In Set II, whilst the lice showed a preference for violet and indigo, they behaved irregularly toward the other colours, this being attributable probably to their wandering owing to hunger. The experiments with lice upon a polychrome carpet afforded no evidence of a preference for any particular colour on the part of the insects.

2. THE INFLUENCE ON LICE OF TEMPERATURE CONDITIONS IN CLOTHING AND THE ABSORPTION OF RADIANT HEAT BY CLOTH.

Galli-Valerio (xii. 1916, p. 35) was obviously wrong in attributing the reported inimical effect of black clothing to colour, for black is not a colour. *A priori* it appeared to me most probable that the effect would be found to depend upon the greater power of absorbing radiant heat possessed by black clothing when compared to that made of various coloured fabrics, it being a matter of common knowledge that black clothing may be intolerably hot when worn by persons exposed to the sun. White is worn in the tropics or summer because it reflects the heat rays that are to a greater or less extent absorbed by black and coloured fabrics.

The difference in the heat-absorbing capacity of clothing materials was studied by Krieger[1], who found that white fabrics (cotton, wool, etc.) acted very similarly. When their behaviour was compared to that of various coloured or black materials, a distinct difference was observable. If a white fabric be taken as 100, the following showed an ascending scale of absorbing power for radiant heat: pale sulphur yellow 102, dark yellow 140, light green 155, dark green 168, turkey red 165, light blue 198, black 208.

In this connection, I would refer the reader to *Parasitology*, x. pp. 89–92, 132, wherein the climatic conditions prevailing in clothing are considered and it is shown that a rise in temperature to ca. 35° C. may be distinctly inimical to lice (30–32° C. being favourable), that owing to the high temperature near the body in summer the insects tend to wander out upon ordinary garments, and that for the same reason they wander away from persons in fever. Moreover, it has been noted (*Ibid.* pp. 90, 579) that persons leaving temperate climates for the tropics may become freed from body-lice.

Experiments.

A double thickness of black serge was cut in the shape of a circle, one-half was covered and the other lined with white linen and the whole stitched together to form a mat fitting into a glass dish which rested on a black background. A number of lice were distributed evenly over the bottom of the dish and exposed to the sun's rays after the mat had been placed over the lice. Two experiments were made on a warm day with a total of 54 lice:

[1] Cited by Kratschmer in *Weyl's Handb. d. Hyg.* I. p. 379, Jena, 1896.

on the side (*a*) where the black faced the sun, after 15 and 30 minutes' exposure, but 3 lice were found, 51 having wandered to side (*b*) where the white faced the sun. Thermometers placed beneath (*a*) and (*b*) registered respectively 47° and 41° C. In short the heat absorbed by the black cloth became intolerable, and the lice had fled to the cooler half of the mat.

When the temperature beneath the mat fell, owing to clouds masking the sun, the two thermometers recording but 24° and 28° or 30° and 33° C. respectively, the lice showed no preference for either half of the mat.

A piece of linen quartered black and white (Fig. 1 *B*) was next used to cover 39 lice exposed to the sun. After 10 minutes, 30 lice were found beneath the white, 7 beneath the black, and 2 were found crawling upon the black. The thermometers registered 38·5° and 31° C. respectively beneath the black and white. In this case, therefore, when exposed to 38·5° C. beneath black, the insects preferred to face the bright light beneath the undyed linen than the higher temperature beneath the blackened portion.

The foregoing experiments demonstrate that black clothing repels lice by virtue of its capacity for absorbing radiant heat.

Conclusions.

Man's clothing, depending upon its colour, varies in its capacity for absorbing radiant heat. In warm weather, black cloth, which absorbs the maximum of heat rays, will prove inimical to lice and cause them to wander away to where it is cooler so as to escape (*a*) the direct effect of heat, and (*b*) its indirect effect in producing perspiration in man, for lice object to excessive moisture in vicinity to man.

3. THE INFLUENCE OF BLACK, WHITE, AND COLOURED BACKGROUNDS AND OF COLOURED SCREENS UPON PIGMENTATION IN LICE, PROVING THAT PIGMENTATION IS NOT HEREDITARILY TRANSMITTED.

(*a*) DEGREES OF PIGMENTATION OBSERVED IN LICE OBTAINED FROM VARIOUS RACES OF MAN.

Murray (1861, p. 571) states that the colour of lice found on different races of man varies, being "nearly black" on West Africans and Australians, dark and dusky on Hindus, orange on Mozambique Africanders and Hottentots, yellowish brown on Chinese and Japanese, dark brown on Andes Indians, dusky olive on Californian Indians, whilst on Esquimaux the lice are paler and more approaching those found on Europeans. He illustrates the tarsi and antennae of specimens obtained from the various races named.

Darwin (1880, p. 169), refers to Murray's paper, and writes that lice from Sandwich Islanders "were darker coloured and appeared different from those proper to the natives of Chiloe, in South America."

As Murray (*Ibid.* pp. 567–571) pointed out, the general colour of lice is due to (*a*) their integumentary pigment, and (*b*) the colour of their gut contents.

Those who regarded whites and negroes as belonging to different species, concluded that the lice found on them were also of different species because of their difference in colour. Murray, like others before him, found that lice taken from negroes were dark whilst those from whites were pale. He remarks that colour is of little value in differentiating species because it "may be derived from the nature of the feeding ground," some observers stating indeed that the negro's lice grow pale when transferred to whites and *vice versa*. The Rev. Hislop, "known as well for his scientific as for his missionary labours in India," informed Murray, "that at Nagpore he thinks he has seen dark Pediculi which have found their way from coloured nurses to white children, after a time becoming white," and another friend told him that "when seated in church behind two lads—the one dark-haired and the other light-coloured, both swarming with vermin—he noticed that those upon the dark-haired lad were darker than those upon the light-haired one."

That *Phthirus pubis* is likewise capable of changing its colour is indicated by Mr G. E. Bodkin (Government Economic Biologist, Science and Agriculture Department, Georgetown, Demerara), who, in a letter addressed to me (8. iv. 1918), states that the insect is very common in Demerara and that he "can speak from personal experience with regard to the chameleon-like qualities of these creatures; when transferred from a negro (where of course they exactly match the colour of the skin) to a white person, they will, in the course of a few generations become almost transparent and extremely difficult to see so closely do they resemble the white skin to which they are attached."

Whereas all specimens of *Phthirus pubis* collected from whites and examined by me have been found to be pale or slightly pigmented, those found on Suaheli negroes (N. 284 from Zanzibar, sent by W. Mansfield-Aders, and other lots) are deeply pigmented in the heavily chitinized regions, the whole integument being dusky.

The accuracy of Murray's statement regarding the variation in colour shown by lice on different races of man has been doubted by several authors. Schjödte (1864) noted the great variability in the colour of lice and so did Neumann (1910), the latter, however, records the presence of dark head-lice on negroes. Piaget (1880, p. 622) collected body-lice in Holland and saw great differences in the coloration of the specimens; he compared lice from Malaya and Europe and found them alike. Blanchard (1890, p. 437) refers to Murray and agrees with him in regarding the colour differences as devoid of specific significance, he nevertheless cites F. A. Pouchet (no reference; probably 1832–1841) as considering lice from negroes and whites distinct species.

Although not referring to Murray, Sikora (viii. 1915, p. 533) notes that variation in colour can be observed in lice taken from one person, three types, white, medium and dark being distinguishable to the naked eye. Peacock (1916, p. 32) examined 1800 lice (source and stages not stated) and found 5·4 per cent. to be "black."

Many authors, in seeking for differences between head-lice and body-lice, lay stress upon the colour. The head-louse is stated to have a dark or cindery gray colour, whilst the body-louse is light or pale coloured (Guérin, 1829–44; Cummings, 1915, etc.) but Schjödte (1864), Piaget (1880), Neumann (1910) and Sikora (1915) reject the colour difference as inconstant. Sikora (1917, p. 172), without giving the reference, cites Fahrenholz as lately having described *Pediculus capitis* var. *maculatus* from negroes, *P. capitis* var. *angustus* from Japanese, and *P. corporis* var. *marginatus* from Japanese, but makes no comments. Judging from Fahrenholz's other publications to which I shall refer elsewhere, this author has also in this instance merely burdened science with three useless names which will fall into the synonymy of *Pediculus humanus*.

Personal Observations.

A survey of some thousands of lice that have reached me from various parts of the world, has revealed that they possess different degrees of pigmentation which can be classified as follows; in the darkest specimens the markings (pleurae, bands, plates, etc.) appear blackish brown and the general integument dusky:

Capitis.

Very dark: N. 257, from Masai negro, German East Africa; N. 76, from negro, Obuasi, Ashanti; N. 244, from East Indian, Madras; N. 240, from Hill woman, Kasauli, India; N. 260, 263, East Indian, British Guiana; N. 231, from Arab, Khartoum, Sudan; N. 249, from native Indian, Lima, Peru.

Very dark to dark (mostly very dark): N. 254, from Suaheli, Zanzibar; N. 243, from East Indian, Madras; N. 229, from Tamils, Federated Malay States.

Dark: N. 235, from Badaga tribe, Nilgiris, India; N. 214, from Arabs, Algeria; N. 268, from Blackfoot Indians, Alberta, Canada; N. 146, from Chinese, Tientsin, China; N. 40, from Eskimo, Frobisher Bay, Baffinland.

Medium: N. 246, from Copper Eskimo, Coronation Gulf Region; N. 176, from Chinese, Szechuen, China; N. 226, from native children, Salonika and Seres.

Medium to pale: N. 208, 282, from grey-haired women (hair partly black or brown), lice mostly medium; N. 211, from soldier's pubic hair (brown), lice mostly medium; N. 181, 209, from children, mostly medium. All of these lice were taken from Europeans.

Corporis.

Dark: N. 225, from negroes and their clothes, Kibondo, Belgian Congo.

Pale to very dark (mostly pale): N. 216, from negroes' clothes, Nigeria; N. 277–281, from negroes, Nairobi, British East Africa.

Pale to dark (mostly pale): N. 217, from Negroes' blankets, Nyasaland; N. 269, from Blackfoot Indians, Alberta, Canada; N. 165, from Chinese, Szechuen, China; N. 213, from Arabs, Algeria. From Americans' and Euro-

peans' clothing: N. 228, Minneapolis, Minn., U.S.A.; N. 33, Liverpool; N. 204, Lambeth; N. 212, London; N. 252, Cambridge.

Pale to medium: N. 259, 261, 222, from East Indians, British Guiana; N. 245, from East Indian, Madras; N. 230, from Sudanese, Khartoum; N. 91, from negro, Biskra, Algeria; N. 45, from Japanese, Yokohama; N. 234, from Budaga tribe, Nilgiris, India.

Pale to slightly pigmented: N. 270, from Suaheli native, Zanzibar; N. 241, from Samper boy, Kasauli, India; N. 232, from Arab, Khartoum, Sudan; N. 90, from Arab, Biskra, Algeria.

Pale: N. 250, from native Indians, Cerro de Pasco, Peru; N. 233, from Arab, Khartoum, Sudan; N. 179, from Chinese, Szechuen; N. 40*a*, from Eskimo, Frobisher Bay.

NOTE. I am indebted to the following gentlemen for collecting or procuring the specimens mentioned in the foregoing list, relatively few having been collected by me personally: Messrs W. Mansfield Aders, G. E. Bodkin, A. J. Chalmers, J. W. Cornwall, J. Donovan, L. Dudgeon, Dr Graham, W. H. Hamer, F. Harker, C. Gordon Hewitt, F. Johansen, W. A. Lamborn, J. W. S. Macfie, J. L. Mitter, A. Owston, R. E. Ribeyro, W. Rose, P. H. Ross, G. G. Sampson, Ed. Sergent, W. R. Sheriffs, S. A. Stericker, C. Strickland, G. L. Tuck, A. M. D. Turnbull.

(*b*) EXPERIMENTS WHEREIN *P. HUMANUS* WERE RAISED ON BLACK, GRAY, WHITE AND COLOURED BACKGROUNDS.

Doubtless acting on the suggestion contained in Murray's paper above cited, Sikora (v. 1917, p. 172) carried out experiments regarding the influence of black, gray, and white backgrounds upon pigmentation in body-lice (*corporis*). In a subsequent paper, Sikora (IX. 1917, pp. 178–179) records similar experiments with head-lice (*capitis*). I have but recently been able to consult this author's papers, which possess considerable interest as throwing further light on the subject. Sikora states that lice maintained on black, gray, and white backgrounds become correspondingly coloured, irrespective of whether they are kept in darkness or exposed to light.

In this connection I have carried out the following experiments in Cambridge:

On 17. v. 1918, Mr A. Bacot kindly sent me some *corporis* (N. 265, 4 ♂, 6 ♀) which had laid some eggs on gray flannel in one of his breeding boxes. The specimens were either pale or moderately pigmented.

Experiment I (Black and White).

As the eggs hatched out (24–30. v. 18) the unfed larvae were placed daily in equal numbers in two pill-boxes so that finally each box contained 46 larvae. The pill-boxes were arranged as described by me in *Parasitology*, X. p. 107, with the difference that the one (*a*) was painted black inside with India ink,

the other (*b*) being left white, whilst instead of a hair-grid, a narrow strip of cloth was wound inside against the sides of the box. The white box contained white flannel, the black box contained black cloth, and its gauze top was blackened. The lice were fed twice daily on my Laboratory Assistant's arm, and, between feeds, were maintained at 31° C. in a glass-doored thermostat facing a window. But little light penetrated the boxes through their upturned gauze fenestra and the insects were disturbed as little as possible. The adults began to emerge in both boxes on the same day (5. vi. 18), and 77 out of 92 reached maturity, the black box containing 40 (18 ♂, 22 ♀) and the white box 37 (16 ♂, 21 ♀). The results in respect to pigmentation were as follows:

(*a*) *Black box*: all the adults (40) were very darkly pigmented, almost as dark as specimens of *capitis* collected from African negroes. The whole integument appeared ashy, whilst the heavily chitinized structures (sides of thorax, pleurae, plates, dorsal bands of male, etc.) were blackish brown and the head and legs dark. (See Plate X, fig. 1.)

(*b*) *White box*: all the adults (37) were pale, whitish or translucent, a few females showed moderately pigmented genital plates, sides of thorax and heads, but almost all had colourless pleurae and legs. (See Plate X, fig. 2.)

The eggs laid by the lice in boxes (*a*) and (*b*) were left *in situ*, hatched out at 31° C., and the larvae raised to adults without being disturbed (see Expt. III). The black adults from box (*a*) were transferred on 11. vi. 18 to white boxes and the pale adults (*b*) to black boxes (see Expt. IV).

Experiment II (Black, white, gray).

The same parent lice that were used in Expt. I, to the number of 2 ♂♂ and 4 ♀♀ were allowed to lay six batches of eggs in as many pill-boxes. Three boxes were placed fenestrum up in the thermostat whilst three stood bottom up with the gauze resting on the floor of the thermostat or on black cloth to exclude the light.

A. Boxes with gauze fenestra uppermost.

(*c*) *Black box*, contained 30 eggs laid 22–23. v. 18. Hatching began 29. v. and 16 adults emerged 8–11. vi. Examined 6–8 days after moulting, 15 appeared dusky and darkly pigmented, and 1 ♀ was moderately dark.

(*d*) *White box*, contained 31 eggs laid 23–24. v. 18. Hatching began 30. v. and 19 adults emerged 9–11. vi. Examined 6–8 days after moulting, 17 insects appeared pale and 2 females showed slight pigmentation at the sides of the thorax and pleurae.

(*e*) *Gray box*, contained 27 eggs laid 24–25. v. 18. Hatching began 31. v. and 19 adults emerged 12–16. vi. Examined 6–10 days after moulting, the colouration of each individual being separately noted with reference to a colour scale, the insects showed various grades of pigmentation. A few were very slightly pigmented, the majority moderately pigmented, but none were as dark as those in black box (*c*).

B. Boxes with fenestra undermost (partial darkness).

(*f*) *Black box*, contained 25 eggs laid 25–26. vi. 18. Hatching began 2. vi., and 7 adults emerged 11–13. vi. Examined 4–6 days after moulting, 6 of the adults appeared dark and 2 moderately pigmented.

(*g*) *White box*, contained 30 eggs laid 26–27. vi. 18. Hatching began 2. vi., and 11 adults emerged 11–14. vi. Examined 3–6 days after moulting, 8 adults appeared pale and 3 slightly pigmented.

(*h*) *Gray box*, contained 32 eggs laid 28–29. vi. 18. Hatching began 3. vi., and 7 adults emerged 14–17. vi. Examined 5–8 days after moulting, the insects (as checked by the colour scale) appeared as a whole slightly darker than those in lot (*e*).

Possibly owing to lack of ventilation, the number of lice raised to maturity in boxes B (*f–h*) was smaller than in boxes A (*c–e*), i.e. 25 from 87 eggs as compared to 54 from 88 eggs. Otherwise there was no material difference in the rate of development. In Expts. III–VI that follow, the boxes were stood in the thermostat with their fenestra uppermost.

Experiment III (second generation on black and white).

The eggs laid (*a*) in the black box by dark adults, and (*b*) in the white box by pale adults in Expt. I, were maintained in said boxes. In the black box, hatching began 15. vi. and adults began to emerge 26. vi. In the white box, the corresponding dates were 16. vi. and 28. vi. The hatching period was one day longer in the white box and the developmental period (1st larva to adult) was two days longer than in the black box. The adults in the black box were very dark, those in the white box pale, the moulted skins of the immature stages being correspondingly dark and pale. These lice, representing the second generation raised on black and on white respectively, were neither darker nor paler than specimens of the first generation similarly raised.

Experiment IV (transferring dark lice to white and *vice versa*).

The dark adults of Expt. I were transferred to a white box and the pale adults to a black box on 11. vi. 18, where they laid eggs that began to hatch 19. vi. The adults began to emerge 31. vi. The progeny of the pale lice raised on black were dark throughout and *vice versa*.

Experiment V (black and white).

Adults raised on black cloth and consequently dark, to the number of 2 ♂♂ and 3 ♀♀, were placed on 2. vii. 18 in each of six boxes arranged as described below. The adults laid eggs which began to hatch 10. vii., and the adult offspring began to emerge 21. vii. The offspring were examined on 23. vii. and all the moults of the six lots collected and separately mounted on slides.

Box No.	Outside of box and gauze	Inside of box	Cloth		Lice found in the box	Pigmentation
1	black	black	black	23	(5 ♂, 5 ♀, 13 L.)	●●●●●
				5	(5 L.)	●●●
					moults	●●●●● to ●●●●
2	black	white	black	22	(7 ♂, 6 ♀, 9 L.)	●●●●
				12	(4 ♂, 2 ♀, 6 L.)	●●●
					moults mostly	●●●●, few ●●●●● few ○
3	black	white	white	25	(6 ♂, 9 ♀, 10 L.)	○
				9	(1 ♂, 1 ♀, 7 L.)	● to ●●●
					moults mostly	○○, few ●●●
4	white	white	white	19	(4 ♂, 1 ♀, 14 L.)	○
				2	(1 ♂, 1 L.)	●
					moults	○, but 2 ●●●
5	white	black	white	17	(6 ♂, 5 ♀, 6 L.)	○
				14	(8 ♂, 1 ♀, 5 L.)	●●●
				3	(1 ♂, 2 L.)	●●●●
					moults mostly	○, few ●●●
6	white	black	black	31	(7 ♂, 7 ♀, 17 L.)	●●●●●
				3	(3 L.)	●●●
					moults	●●●● to ●●●

NOTE. The degrees of pigmentation are indicated as follows in the foregoing and succeeding records: ●●●●● = very dark, ●●●● = dark, ●●● = medium, ● = slight, ○ = pale to very pale.

Experiment VI (red and yellow).

Adults of the same lot as in Expt. V were put to lay (1. vii. 18) in two boxes with the inside, outside, gauze and contained cloth coloured (*a*) pale yellow with picric acid and (*b*) bright red with ink. The eggs began to hatch on the ninth day on yellow, on the eighth day on red, and the adults emerged 11 days later. The examination on 23. vii. revealed the following:

			Pigmentation
Yellow box contained	24	(6 ♂, 7 ♀, 11 L.)	○ or ●
		moults	○ or ●
Red box contained	35	(13 ♂, 7 ♀, 15 L.)	●●●●●
	2	(2 L.)	●●●
		moults	●●●●● to ●●●●

Experiment VII (lice on white, in complete darkness).

Dark adults of the same lot used in Expt. V, were put to lay on 2. vii. 18 in two boxes: (*a*) all black (like box 1, Expt. V) and (*b*) all white (like box 4, Expt. V). These boxes were enclosed in thick, tightly closing wooden boxes blackened inside and outside with India ink. The boxes were opened twice daily in the dark for the purpose of feeding the adults on three successive days during which they oviposited. The adults were then removed, and the boxes with eggs were returned to the thermostat for six days. After this, the hatching having commenced, the young lice were fed twice daily, the boxes containing them being applied to the arm in the dark room, the arm being swathed in

several thicknesses of camera cloth (black, lined red) during the half-hour feeding periods, when my laboratory assistant left the dark room. The lice were exposed to diffuse daylight for only a few minutes as nits (when the parents were removed), and once for 3–4 minutes when the box was inspected to see if the adults had emerged. The result was as follows when the insects were removed on 23. vii. 18:

			Pigmentation
(*a*) Black box (kept in partial darkness) contained	8	(3 ♂, 5 L.)	●●●●
	11	(3 ♂, 4 ♀, 4 L.)	●●●
	1	(♂)	●
		moults	●●●● and ●●●
(*b*) White box (kept in complete darkness) contained	34	(5 ♂, 14 ♀, 15 L.)	○ to ○○

N.B. Expt. VII *a* was vitiated by the entrance of light. It was intended that it should be carried out in complete darkness. Owing to the dry atmosphere in the thermostat, a minute pin-point crack developed in the lid of the outer wooden box, and light entered in sufficient amount to affect a small central area on photographic paper placed in the bottom of the box.

Experiment VIII (rate at which lice darken on black).

Second and third stage larvae that had been raised from the egg in a white box on white flannel and which were consequently pale or but slightly pigmented, were transferred on 25. vii. 18 to a black box containing black cloth. After 36–48 hours had elapsed, 5 adults had emerged and they were dark or moderately pigmented. On the day following some more dark adults and dark third stage larvae were found in the black box. Diffuse light entered the box whilst it was in the thermostat.

Experiment IX (black and white. Lice in absolute darkness).

On 29. vii. 18, seven pale third stage larvae were placed on black cloth in a black pill-box enclosed in a black wooden box as in Expt. VII, it being first ascertained that the latter was light tight. As an extra precaution, these boxes were enveloped in black cloth and the bundle placed in a third box. The lice were fed twice daily in a photographic dark room and were kept at 31° C. during the intervals between feeds. The insects were maintained inside the triple boxes except when being fed in the dark room through the gauze top of the innermost box.

The adults emerged on 30. vii. and all of them from the black as well as the white box were pale.

Experiment X (black and white. Lice in a bright light).

A number of first stage larvae were placed in two vertical glass tubes each plugged below by a cork upon which was spanned in the one case white linen in the other black sateen. They were exposed to bright light day and night for about two weeks. (The experiment ran concurrently with Expt. II as

described on p. 216.) Of the seven adults raised on white four were pale, two very slightly and one slightly pigmented. But two adults were raised on black, one was dark and the other pale, the larvae that died whilst being raised were frequently darkly pigmented. The heat absorption by the black affected the insects adversely.

Experiment XI (different colours).

On 10. viii. 18, second and third stage larvae of the foregoing strain of lice were removed from an overstocked breeding box which contained white flannel that the insects had soiled. It was noticed that many larvae were darkly pigmented and this was attributed to (1) the lice being crowded, and (2) the box being soiled. These larvae were distributed in differently coloured boxes (tabulated below) and fed twice daily. The boxes were opened once every 24 hours to remove any adults that had moulted, these insects being isolated in boxes of corresponding colours for 24 hours at 31° C., thus giving them plenty of time to darken. The following results were recorded in respect to pigmentation:

Colour of box	August 12	13	14	15	16	17–18	19
violet	.	.	1 ●●●● 1 ○	3 ●●●●	4 ●●●●●	1 ●●●●	.
indigo	1 ●●	1 ●	1 ●●●●● 1 ●●●	1 ●●●●	2 ●●●●● 3 ●●●	.	.
blue	.	.	.	2 ●●●● 1 ●●●	1 ●●● 1 ●●	1 ●●●● 1 ●●●	.
green	.	.	1 ● 1 ○	2 ●● 2 ○○	1 ●● 1 ● 2 ○	.	.
orange	.	.	2 ●● 1 ● 1 ○	1 ●●●● 2 ●● 1 ○	.	.	.
black	.	.	.	.	1 ●●● 1 ●	2 ●●●● 1 ●●● 1 ●● 1 ●	2 ●●●●

Summing up the results, the different boxes yielded:

violet	10	(7 ♂, 3 ♀)	4 ●●●●● 5 ●●●● 1 ○
indigo	10	(4 ♂, 6 ♀)	3 ●●●●● 1 ●●●● 4 ●●● 1 ●● 1 ○
blue	7	(4 ♂, 3 ♀)	3 ●●●● 3 ●●● 1 ●●

green	10	(7 ♂, 3 ♀)	1 ●●
			4 ●
			5 ○
orange	8	(6 ♂, 2 ♀)	1 ●●●●
			4 ●●
			2 ●
			1 ○
black	12	(6 ♂, 5 ♀, 1 ♀̂)	4 ●●●●
			5 ●●●
			1 ●●
			2 ●

The adults that emerged on green (especially) and on orange, were paler than the rest with few exceptions. Those on violet, indigo and blue would appear to have become progressively darker the longer they sojourned in their boxes prior to moulting. On the other hand the previous sojourn in the soiled stock box appears to have exerted an after effect on the insects kept on orange, if we compare the results with those of the following experiment.

To exclude any possible after effects of a previous sojourn on a background which would induce darkening, the following experiment was carried out.

Experiment XII (different colours).

Lots of 10 first stage larvae, the progeny of pale parents in a clean white box, were put in coloured boxes (tabulated below) on 23. ix. 18. On blue, violet and black, the first adults emerged on 3. x., and the last on 5. x. On green, indigo, yellow and orange, the first adults emerged on 4. x. and the last on 6. x., consequently there was no distinct difference in the rate of development.

Colour of box			Pigmentation in adult lice raised
violet	8	(6 ♂, 2 ♀)	7 ●●●●
			1 ●●●
indigo	7	(5 ♂, 2 ♀)	4 ●●●●
			3 ●●●
blue	8	(7 ♂, 1 ♀)	5 ●●●●
			2 ●●●
			1 ○
green	8	(6 ♂, 2 ♀)	1 ●
			7 ○
yellow	10	(6 ♂, 4 ♀)	10 ○
orange	10	(4 ♂, 6 ♀)	2 ●●●
			8 ○
red	10	(8 ♂, 2 ♀)	10 ●●●●
black	7	(7 ♂)	9 ●●●●
			1 ○ (killed young)

This shows more distinctly than Expt. X that lice raised on green, yellow, and orange do not become pigmented like the others.

THE INFLUENCE OF COLOURED RAYS OF LIGHT ON PIGMENTATION IN LICE MAINTAINED ON WHITE FABRICS.

Having established that lice do not become pigmented when maintained in absolute darkness, and that exposure to a very small amount of light suffices for them to become pigmented if maintained on black and red backgrounds, etc., it seemed of interest to observe if coloured rays of light exert an influence on pigmentation.

Method. Seven cylindrical glass specimen jars, ca. 12 cm. high by 5 cm. in diameter were partly filled with solutions of the seven dyes enumerated on p. 203. The jars were closed by corks of suitable size bored centrally to hold short test tubes which were pushed through up to the rim. The tubes thus became surrounded by a uniform layer of dye solution except at the neck. The jars were stood in alcoves made of cardboard boxes covered with white paper and facing the glazed door of the thermostat opposite a window, the tubes being capped by a piece of white cardboard. Each tube contained a strip of white flannel (ca. 3·5 × 0·7 cm.) standing vertically inside, to which the lice (second and third stage larvae, 10 per tube) that were introduced could cling. The lice were fed in pill boxes whose interior was coloured with the corresponding dye solutions to those contained in the jars, the insects being transferred to the boxes on the pieces of flannel.

Experiment I. Pale lice were maintained for 48 hours in the apparatus; they moulted therein and all of them were found to be pale or very slightly pigmented. Under the conditions of the experiment the coloured rays therefore appeared to exert no effect. The experiment was therefore repeated and varied, the insects being maintained a longer time exposed to coloured rays.

Experiment II. Newly emerged first-stage larvae were placed in the apparatus and raised to maturity whilst *exposed continuously to light.* During the day-time the light emanated from the window and during the evening and night from a 60-candle power electric lamp suspended close to the glazed door outside the thermostat. To ensure a maximum of illumination, a looking glass was placed at the back of the thermostat to reflect the infalling rays into the tubes that contained the lice, whilst the inside of the thermostat was lined with white paper. A piece of starched white cotton netting was placed in each tube for the lice to cling to in lieu of white flannel because the latter afforded a slight amount of shade. The experiment lasted two weeks.

The adults were carefully graded in respect to degrees of pigmentation and classified as follows, the sign × denoting separate individuals:

Colour of tube	Total adults raised	Pigmentation				
		dark	medium	slight	very slight	absent
Violet	9	×	× × ×	× ×	× × ×	.
Indigo	10	.	× × ×	× × ×	× × × ×	.
Blue	8	×	×	×	.	× × × × ×
Green	9	.	× ×	×	× ×	× × × ×
Yellow	11	.	.	×	.	× × × × × × × × × × ×
Orange	12	.	.	×	× ×	× × × × × × × × × ×
Red	9	.	×	×	×	× × × × × ×

CONCLUSIONS.

Section (*a*).

The most darkly pigmented *capitis* are derived from dark-skinned black-haired peoples. Head-lice tend to grow paler on yellow or moderately pale races possessing black hair. The palest head-lice are found on white races whose hair is often light in colour.

Whilst the darkest *corporis* are obtained from negroes, the insects in most cases cannot be grouped according to the skin colour of the host. The occurrence of pale body-lice on dark-skinned races is doubtless attributable to their wearing white or light coloured clothing especially in hot countries.

Phthirus pubis derived from negroes are darkly pigmented, whereas those derived from whites are pale for the same reasons that apply to head-lice.

The statement that lice may change colour when transferred from light to dark races and *vice versa* (Murray, Bodkin), in the absence of experiments on man, finds support amounting to confirmation in the experiments herein described wherein lice were raised on differently coloured, black, and white backgrounds.

It appears highly probable that paler head-lice will be obtained from light-haired than from dark-haired individuals among white races. I am at present collecting data in this connection and shall be grateful to any readers who may supply specimens or further information on the subject.

Note. The paper by Sikora (ix. 1917, pp. 278–279) has come to hand whilst this publication is going through the press. According to Sikora, *capitis* derived from black-haired persons are not always darker than those found on individuals with moderately fair hair, but the very fair haired always harbour distinctly paler lice. The contrast in the pigmentation of such lice is never so great as in the insects raised experimentally on black and white respectively. A very blond girl, bearing many pale head-lice, also harboured a few dark lice which were evidently derived from her (verminous) dark-haired mother.

Section (*b*).

The experiments detailed in this section show:

That pigmentation in lice is dependent upon the nature of the background upon which the insects are raised. If eggs laid by dark lice on white cloth are allowed to hatch and develope on white cloth, they will appear pale in all stages from the first larva to the adult (Expts. IV, V). Conversely, if eggs laid by pale lice on black cloth hatch and the insects be raised on black cloth, they will appear dark in all stages. Lice raised on a gray background show various degrees of pigmentation that are intermediate compared with the results obtained on black and white (Expt. II). It is immaterial if the pill-boxes in which the lice are raised are black or white externally (Expt. IV). The darkest lice were obtained in boxes blackened inside and containing black cloth, they were mostly less dark in boxes that were white inside and contained

black cloth (Expt. IV). Conversely, the palest lice were obtained in boxes that were white inside and contained white cloth, a few showed slightly increased pigmentation when the box was black inside and the cloth white (Expt. V).

Lice raised in the light on violet, indigo, blue and red, were as dark as those raised on black (Expts. VI (red), XI, XII), whilst those raised on green, yellow, and orange (Expts. VI (yellow), XI, XIII) were pale or slightly pigmented in the order yellow (acting like white), green and orange.

Lice kept in absolute darkness on black and white, do not show pigmentation (Expt. IX), but when maintained on black, a very small trace of light admitted to them causes pigmentation to take place (Expts. VII *a*, II *B*).

Pale larvae sojourning on black for 36–48–72 hours prior to moulting, show progressive degrees of pigmentation (Expt. VIII).

Dark adults transferred to a white background and pale adults transferred to a black background do not subsequently undergo modifications in pigmentation. Their coloration is fixed for life.

When many lice inhabit a white box, the colour of their bodies, gut contents and excreta darken the background with the result that the insects kept therein become more or less pigmented (Expt. XI and repeated experience).

Contrary to the experiments with variously coloured backgrounds, those in which lice were exposed on white flannel for 48 hours to the action of coloured rays of light did not show differences in so far as all remained pale or very slightly pigmented.

A longer exposure to coloured rays exerts an influence on pigmentation, for lice exposed continuously to coloured light whilst being raised to maturity were, as a whole, decidedly darker in the violet and indigo tubes than in the others. There were intermediate in the blue and green, and mostly pale in the yellow, orange and red tubes.

The experiments explain the great variation in the pigmentation of lice found on different races of man and variability in the pigmentation of lice occurring on white races, the colour of the skin, hair, and clothing, all exerting their effect on the insects. The statements are doubtless correct (*a*) that dark lice from negroes may turn pale when transferred to whites and *vice versa*, and (*b*) that head-lice on dark-haired whites are darker than on the fair-haired. The latter statement is being investigated.

The change in pigmentation of lice, due to the colour of the background, doubtless affords a measure of protection to these parasites in nature, especially in the case of man, who, like the monkey, uses his eyes in the search for the insects.

The observations recorded in Section (*a*), but especially in Section (*b*) show that Hindle (*Parasitology*, IX. p. 265) was unfortunately misled in his experiments designed to breed pigmented and unpigmented strains of *P. humanus*. Hindle readily reared colourless strains but had difficulty with dark ones, and

concluded that "there seems no doubt that the method of inheritance is alternative, but whether associated with the sex or not is not yet decided."

Pigmentation in *Pediculus* is not an hereditarily transmitted character, its presence depends entirely upon the nature of the background upon which the insect lives and it is a character that may be acquired in a couple of days (see especially Expts. IV and VIII). This phenomenon should be borne in mind by investigators engaged in the study of heredity.

4. THE RELATIVE PROPORTION OF THE SEXES.

In *Parasitology*, x. pp. 114–115, reference was made to the relative proportion of the sexes in *P. humanus* encountered (*a*) naturally upon the host and (*b*) in broods raised experimentally, and it was stated that further enumerations were required before reaching final conclusions on the subject. An examination of Bacot's hybrids of *capitis* and *corporis* has revealed the presence of a large number of hermaphrodites amongst them, a discovery that vitiates the figures cited (*loc. cit.*) from that author. A detailed study of these and other hermaphrodites will form the subject of another paper by Dr Keilin and myself.

ENUMERATION OF SEXES IN *PEDICULI*:

(*a*) *in insects encountered naturally upon the host.*

The following enumerations are based on specimens which have reached me from various parts of the world. The different lots contained numerous immature stages together with the adults.

No. of lot	Description	Total adults	♂	♀	♀̂	% ♀
273	*corporis*, Sydney, N.S.W.	196	62	134	19	68
278	,, Nairobi, B.E.A.	364	141	223	1	64
279	,, ,, ,,	350	131	219	0	63
277	,, ,, ,,	245	95	150	2	61
274	,, Sydney, N.S.W.	103	40	63	0	61
257	,, Tanga, E.A.	173	71	102	2	59
236	*capitis* + *corporis*, Nairobi	358	148	210	0	59
237	,, ,, ,,	214	94	120	2	56
252	*corporis*, Cambridge	446	220	226	0	51
208	*capitis* ,,	208	104	104	0	50
282	,, ,,	35	6	29	0	*
209	,, London	39	14	25	0	*
285	,, Bogota, Colombia	38	7	31	0	*
	Totals	2769	1133	1636	26	59%

* These figures alone are too small to afford a reliable percentage. 0·5 % or over is reckoned as 1 % so as to give a round figure in the percentage column.

(*b*) *found in broods raised experimentally.*

The following enumerations are based on one lot (N. 265) of *corporis* raised in successive broods in Cambridge during August–October 1918 from a few parents kindly supplied by Mr A. Bacot. The breeding boxes contained black cloth, the insects were fed twice daily and maintained at 31° C. in the thermostat between feeds.

Date of emergence	Total adults	♂	♀	% ♀
Prior to following	357	167	190	53
" "	149	66	83	56
21. x.	210	136	74	35
22	71	44	27	*
23	31	18	13	*
28	80	46	34	*
30	54	24	30	*
Totals	952	501	451	47 %

* See footnote to previous table.

CONCLUSION.

In my previous paper (*loc. cit.*) it was stated that of 208 adult *capitis* collected by me from a woman in Cambridge 50 % were female; of 100 *capitis* raised by me 57 % were female, whilst of 944 *corporis* raised by Hindle roundly 60 % were female. (The first of these records is included in the table on p. 219.)

Of 2769 adult *corporis* and *capitis* collected for me in different parts of the world 59 % were females. Of 952 *corporis* raised by me experimentally in Cambridge 47 % were females.

It would appear from the tabulated figures that the proportion of the sexes varies, this depending on conditions which remain to be determined.

SUMMARY.

Refer to the conclusions stated at the end of the foregoing sections on pp. 204, 206, 217–219 and the foregoing paragraph.

REFERENCES.

See Bibliography in *Parasitology*, x. pp. 1–42, 582–586.

Sikora, H. (ix. 1917). Zur Kleiderlaus-Kopflausfrage Vorläufige Mitteilung. *Arch. f. Schiffs- u. Tropen-Hyg.* xxi. 275–284, 3 figs.

Corrections.

P. 467, 468, 475, Pl. X, figs. 1-2. *The "Newman" Disinfector* was designed and an illustrated description of it published by Dr John C. Thresh in 1902 (see The "Emergency" Disinfector, *Brit. Med. Journ.* i. pp. 1606–1608, figs. 1, 2). Dr Thresh informs me that the perusal of my paper (*loc. cit.*) gave him the first intimation that his apparatus was being sold under the Trade name of the "Newman." The vendors of the latter might with advantage give credit to the designer in the circulars dealing with this apparatus.

P. 426, footnote. *Read* "Semon" *in place of* "Lemon." The error is due to a misprint in the publication quoted.

P. 581. "duddie" should be defined as meaning "ragged."

EXPLANATION OF PLATE X.

Pediculus humanus corporis. Specimens treated with caustic potash and mounted in balsam. Illustrating Experiment I (see text, pp. 209–210).

Fig. 1. 3 ♂ and 7 ♀ (10 out of 40 raised) maintained in a black pill-box. All the insects were darkly pigmented.

Fig. 2. 8 ♂, 7 ♀ (15 out of 37 raised) maintained in a white pill-box. Nearly all the insects are pale or but slightly pigmented.

THE COMPARATIVE EFFECTIVENESS OF CERTAIN CULICIFUGES UNDER LABORATORY CONDITIONS.

By A. BACOT,

Entomologist to the Lister Institute of Preventive Medicine,

and Private G. TALBOT, R.A.M.C.

(With 1 Text-figure.)

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I. INTRODUCTION.

The methods and experiments detailed in the following paper were devised for the purpose of determining for the War Office the comparative efficiency of certain Culicifuges, the work being carried out at the Lister Institute of Preventive Medicine.

Any preparation which will repel the attacks of mosquitoes, if only for a short period, is a desideratum in countries where these insects abound. In the case of soldiers in malarious countries, an effective culicifuge would be of great importance, as troops are necessarily exposed to risks not incurred by civilians, as, for instance, outpost and sentry duty at night, which debars the free use of mosquito curtains and at the same time restricts freedom of movement. In our opinion the experimental results obtained are sufficiently promising to justify their publication, together with the methods by which they were attained, in the hope that future work on these lines will establish a scientific basis for the use of culicifuges.

The bulk of the preparations were made up by Messrs Burgoynes from formulae collected by the War Office; these include Nos. 1–6, 9 *a*, 15 *a* and 16.

In addition, certain proprietary articles, Nos. 7, 10–14, 17, 19 and 22, recommended as efficient in keeping mosquitoes and midges at bay, were tested as well as a few preparations made up by the authors. As regards these latter the reasons for their inclusion are varied. Nos. 8, 9 and 9 *b*, variants of 6 and 9 *a*, were compounded with a view to checking the effect of altering the relative proportions of the active constituents and base. No. 15 was tried on account of its general high value as an insecticide; the formulae given being devised for another insecticidal purpose. Subsequently it was put up specifically as a culicifuge—No. 15 *a*. Nos. 18 and 20 were given a trial on account of recommendations, while the inclusion of 21 was due to its time-honoured use by fishermen and field naturalists.

After making due allowance for the variable conditions and divergent numbers of mosquitoes present in the cages in the several tests, we suggest that our experimental evidence clearly discriminates between the several preparations tested; it shows that, while some afford a high degree of protection, others are of dubious value.

Without committing ourselves to the conclusion that the value of these preparations in the field against *Anopheles* mosquitoes will be exactly parallel to their use against *Stegomyia fasciata* (*Aëdes calopus*) under laboratory conditions, we trust that the work will afford a practicable comparative guide. On the other hand we fully appreciate the fact that from the point of view of pure science, we have dealt with only the fringe of a large subject, whilst as regards the practical issues, a really efficient culicifuge, one that will protect continuously for several hours, has yet to be found.

It is very desirable that further investigation should be made concerning the nature of the attraction possessed by man for the female mosquito, and the influence exercised by the culicifuge in combating it.

II: THE CHOICE OF MOSQUITO.

The choice of *Aëdes calopus* Meigen (= *Stegomyia fasciata* Fabr., *Aëdes argenteus* Poiret) for use in these experiments was due to the possession of a West African stock of this species by one of the authors, and by the fact that it can be easily reared in captivity. The females are renowned for their blood-sucking proclivities, feeding just as readily under laboratory conditions as when at liberty, the season of the year making no difference to either breeding or feeding, provided that the factors of the temperature and humidity are favourable.

III. METHOD OF REARING THE MOSQUITOES.

The eggs, laid on strips of filter paper, were immersed in tepid water to which was added a small quantity of brewers' yeast, and placed in an incubator at 95° F. to hatch. After hatching they were transferred to another incubator kept at 80° F. as the higher temperature was probably responsible for the production of weakly adults.

The food given to the larvae—brewers' yeast, pressed out of its wort—was chosen because it gave excellent results in speedy growth; further it was thought that there would be less danger of a poisoned bite occurring if a known organism were present in excess, than would be the case if a nutrient substance were added to the water, and the growth of organisms left to chance.

The only precaution needed with this food was to adjust the quantity of yeast to that of the water and number of larvae present. If too much is added, bacteria multiply to such an extent that a scum is produced on the surface, and is followed by the death of the larvae. The appearance of any scum on the water in the breeding jars should be a signal for a change of water, carried out as follows: The water is strained off through a piece of thin cotton or fine muslin, the jar refilled with fresh water of about the same temperature, and the muslin inverted in it.

For rapidity of rearing it was found more convenient to segregate the pupae and allow the adults to emerge in the incubator at 95° F. than to place the jar containing the pupae in the experimental cage. In order to transfer the adults quickly, a waxed card cream jar, its bottom replaced by gauze, was inverted over the jars containing the pupae, security from falling or slipping off being obtained by a ring of stout paper tied round the neck of the jar, and projecting beyond its edge. The mosquitoes, as they emerged, ascended to the card receptacle. Each morning the stiff paper ring was removed and two pieces of card slipped across the top of the glass jar; the upper card was lifted and placed, with the receptacle containing the adult insects, on a piece of glass, the card withdrawn and the receptacle inverted for the purpose of counting the insects before transference to the experimental cage. This is effected by inserting receptacle and glass through a sleeve attached to a side of the cage (see description in section VII), removing the glass, and tapping smartly the sides of the receptacle to make the mosquitoes fly out.

An equal number of both sexes was transferred to the cages, but by dividing the pupae captured each day between the two jars allotted for the emergence of adults for each cage, the number of females transferred to each can be approximately adjusted.

Further details in regard to the breeding and bionomics of this species of mosquito can be obtained by reference to the report of the Yellow Fever Commission, vol. III (1916).

IV. CONDITIONS NECESSARY TO MAINTAINING A STOCK OF MOSQUITOES.

Our first attempts at keeping a large stock of *Stegomyia* in the cages was not very successful owing to the heavy death-rate, due presumably to the dryness of the atmosphere, and to the numbers which fell into the water receptacles and were drowned. As it was evident that a much more humid atmosphere was required, the top of the cages and three of the sides, which were of gauze, were covered with waterproof paper.

As food for the insects in intervals of blood-sucking, syrup (honey and water) was supplied, as shown in Fig. 1. A flask of water contained a tube formed of several thicknesses of blotting-paper with a piece laid horizontally over the top; on this a little syrup was poured and was kept constantly moist whilst water remained in the flask.

The presence of water to which the mosquitoes have access for drinking and oviposition, is essential to maintain them alive and feeding for any length of time; in order to prevent drowning, dried beech leaves were placed in the tins.

As a certain mortality is sure to occur, a continued supply of adult mosquitoes is necessary to replenish the cages. The eggs which are deposited on the leaves in the tins of water hatch, and the larvae thus obtained will afford a reserve of pupae from which the mosquitoes are bred.

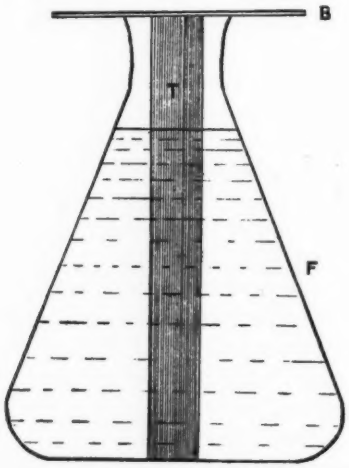

Fig. 1. Arrangement for preventing evaporation of syrup. *F*. Flask containing water. *T*. Tube formed of blotting paper. *B*. Blotting paper to receive syrup, placed over top of flask.

V. DETAILS OF PREPARATIONS TESTED.

No. 1. Oil of Cassia, 1 oz., Brown Oil of Camphor, 2 ozs., Vaseline, Lanoline, or Salad Oil, 3 ozs. (Prepared with wax.)

No. 2. Oil of Peppermint, 1 oz., Oil of Cassia, 2 ozs., Vaseline, Spirit or Pond's Extract, 2 ozs.

No. 2 *a*. No 2 put up with wax.

No. 3. Bamber Oil (Citronella Oil, 1½ pts, Kerosene, 1 pt, Cocoanut Oil, 2 pts, Carbolic 1 %). (Prepared with wax.)

No. 4. Bergamot Oil, 1 pt, Kerosene, 16 pts. (Prepared with wax.).

No. 5. Eucercit (Eucalyptus Oil, 2 ozs., liquid Carbolic Acid, 4 drops, Citronella Oil, 2 ozs.). (Prepared with wax.)

No. 6. Crude Coke Oven Naphthalene, 1 pt, Soft Soap, 3 pts.

No. 7. "Anti-mosquito Cream." (Prepared by Messrs Burgoynes.)

No. 8. Refined Naphthalene and Camphor. Naphthalene 4, Camphor 1, Vaseline 2.

No. 9. Crude Naphthalene (Coke Oven) 4, Camphor 1, Vaseline 2, Paraffin wax ½.

No. 9 *a*. Crude Coke Oven Naphthalene, 3 pts, Camphor, 1 pt, Japan wax, 3 pts, Paraffin wax, 5 pts, Paraffin Molle, 5 pts.

No. 9 *b*. Same as 9 *a* but with 10 pts Naphthalene.

No. 10. "Crude Parasitox." (Prepared by Messrs Heppel.)

No. 11. "Parasitox."

No. 12. "Mosquito Wax." (Prepared by Messrs Lawson and Co.)

No. 13. "Paraquit Wax." (Prepared by Messrs Lawson and Co.)

No. 14. "Paraquit Concentrated Soap." (Prepared by Messrs Lawson and Co.)

No. 15. Light Wood Oil Emulsion. (Light Wood Oil, 19 pts, Soft Soap, 10 pts, Caustic Soda, 5 pts, Water, 1 pt.)

No. 15 *a*. Light Wood Oil, 33 % in a wax preparation.

No. 16[1]. Carbolic Acid, 1 pt, Soft Soap, 3 pts.

No. 17. "Midge Cream."

No. 18. Quinine Sulphate (neutral), 1 gramme, Alcohol, 95 %, 32 c.c., Water, 11 c.c.

No. 19. Saffrol, Chlorobenzene and Chrysarobin, combined with Ethyl Chloride. (Put up by Messrs Hedley and Co.)

No. 20. Light Cedarwood Oil, 2 c.c., Paraffin Wax, 3 grs., Vaseline, about 0·75 gr.

No. 21. Oil of Turpentine, 2 c.c. Paraffin wax, 3 grs., Vaseline, 1 gr.

No. 22. "Lawson's Anti-mosquito Compound."

No. 22 *a*. "Lawson's Anti-mosquito Compound." (Second sample.)

VI. A DISCUSSION OF THE PREPARATIONS TESTED.

Of the preparations tested in Table I, Nos. 1, 3, 4, 5, 6, 8, 9, 12, 13, 15 *a*, 22 and 22 *a* were prepared with wax; Nos. 2, 20 and 21 were of a greasy nature; Nos. 15, 18 and 19 were liquids, and Nos. 10, 11 and 14 were prepared as soap.

It was found that the preparations made up with grease, or as soft soaps, had the advantage in ease and economy of application.

The composition of waxy preparations requires some nicety of adaptation in relation to the temperature in which it is proposed to use them; if this is not done they require warming in order to facilitate spreading, and to obtain an even coat, otherwise they tend to waste in use by flaking off, etc. As regards endurance, however, waxy preparations have a great advantage over greasy or soapy ones, as they form a more permanent covering to the skin, and more effectually retard volatilization. To secure any prolonged protection preparations ought certainly to be made up with wax.

Fluids form a class apart and require much more skill and care in application if waste is to be avoided; this applies more so to highly volatile fluids, as was the case with No. 19.

Soaps, if as hard as Nos. 10, 11 and 14, are best applied by first wetting the skin, otherwise they either coat the skin unequally, or flake off; if, however, they are of the right consistency they should be as easy to apply as grease or soft wax, though they suffer of course from the fact that perspiration will remove soap more readily than it will either wax or grease.

[1] No. 16 was not tested against mosquitoes, as a preliminary trial on a small skin area showed that it caused considerable irritation.

Many culicifuges lack efficiency owing to unsuitable preparation rather than to defects in their essential constituents. There is a general tendency on the part of makers to use too heavy a percentage of some inert base. Though in the case of a wax this may be useful in raising the melting point and in retarding volatilization, it must be condemned where the result is to diminish the effectiveness of the preparations. For instance, preparations 3, 4, 5 and 22 would doubtless give better results if compounded with less base and rendered easier to spread on the skin.

An ideal culicifuge should (1) spread easily so as to enable it to be quickly and evenly applied over the exposed area, while (2) at the same time it must be of such a consistency that it will adhere firmly to the skin, and (3) retard volatilization. In respect of points 1 and 3, preparations need modification according to their intended use in tropical or temperate climates. (See also notes following Table I.)

The individual likes of people in regard to odours are varied and their dislikes so intense in some cases as to amount almost to physical discomfort, with the result that irritation caused by the bites would be preferred to the use of an objectionable culicifuge; some people greatly preferring the heavy odours of spices or gums to the cleaner if more penetrating smells of such substances as naphthalene and tarry or wood distillates.

These considerations make it hard to advise as to the best of a number of preparations giving approximately equal degrees of protection. Among troops and others compulsorily subjected to measures of malaria control, a preparation, the odour of which is greatly disliked, might fail because the majority would not apply it, or do so indifferently. The difficulty might be successfully met by adopting two classes of preparations.

The question of cost is also of importance; where large quantities are needed such preparations as 15 *a*, 9 *b*, and 21 would easily outclass equally serviceable ones depending upon costly essential oils for their efficiency.

VII. METHODS ADOPTED FOR TESTING PREPARATIONS.

The two cages used for carrying out the tests were similar to those employed for experimenting with this mosquito in West Africa under the auspices of the Yellow Fever Commission. The framework is of wood screwed together, the top and sides being of wire gauze with a circular hole 5 inches in diameter cut in one side to which is fastened a sleeve of mosquito netting. These cages measure on the inside 1 foot 6 inches in height, 2 feet in length, 1 foot 3½ inches in breadth. They are fully described and illustrated in the *Reports of the Yellow Fever Commission*, vol. III, pp. 169–172, 1916.

Each cage was used only once in 3 or 4 days, as the mosquitoes' desire for blood normally recurs at about 3–4 days intervals. On the occasions of the tests the mosquitoes were therefore ravenous.

The method adopted in testing a preparation was as follows:

1. The arm, from wrist to elbow, was coated as evenly as possible with the preparation. One gramme was found to be sufficient for the purpose in most cases, and was used as the standard quantity; in a few instances the consistency of the preparation allowed of an economy, so that a few tests were carried out, using ·75 grm. In the case of the liquid preparations, Nos. 18 and 19, larger quantities were necessary, in order to completely coat the desired area of skin.

The hand, during the exposure of the arm in the cage, both in tests and controls, was covered by a rubber glove, it being necessary to restrict the bites to the forearm, owing to the fact that on many parts of the hand the skin reaction is so slight that it is not possible to enumerate bites with accuracy.

In the first series of experiments the arm, after treatment, was covered by the sleeve for 15 minutes to allow of some volatilization, before exposure in the cage for 10 minutes.

In the earlier trials with preparations 1–9 the control exposure of the untreated arm for a similar period, followed immediately after the test. It was found, however, with some preparations, that very few, if any, attacks were made by the insects under these circumstances. This presumably was due to the persistence of the vapour in the cage preventing the mosquitoes from detecting the presence of food. In all subsequent tests, therefore, a longer or shorter interval, usually of from 2–4 hours, was allowed between the test and the control.

2. In the second series of experiments the procedure was the same, except that the arm, after treatment, was exposed to the air for periods of from 2–5 hours before insertion in the cage.

It is to be noted that tests made in the laboratory under the conditions described are really more severe than would be the case were the preparations used in the ordinary way out of doors. Under ordinary circumstances the insects would have a greater range of choice, for even if all the population of a district were similarly protected, there would still be the chance of feeding on other animals; in the laboratory the choice was restricted to a preference for the treated or untreated arm of A. B. or G. T.

VIII. EXPERIMENTS, SERIES I.

Prior to conducting the first series of trials a preliminary census was made in order to ascertain the number of feeding females in the respective cages. On May 21st, 1918, an unprotected arm was exposed in each cage; the number of bites recorded was: Cage A, 41 bites; cage B, 43 bites.

The preparations were tested according to the method already explained. The results are given in Table I.

Table I.

Preliminary Tests of Culicifuges.

(15 minutes after application.)

Preparation used	Quantity	Cage	Date	Room temperature F.	No. of bites on treated arm	No. of bites on untreated arm	Interval between test and control	Tested by
1	1 gr.	B.	24. v. 18	71°	0	30	0	
	·75 gr.	B.	25. vi. 18	66°	0	27	4 hrs.	
2	1 gr.	A.	25. v. 18	71°	0	0	0	
	1 „	A.	4. vii. 18	71°	0	35	5½ hrs.	
3	1 „	B.	27. v. 18	68°	12	6	0	
	1 „	B.	1. vii. 18	73°	4	33	3 hrs.	
4	1 „	A.	29. v. 18	69°	15	5	0	
	1 „	A.	1. vii. 18	73°	18	50	3 hrs.	
5	1 „	B.	31. v. 18	70°	5	16	0	
	1 „	B.	4. vii. 18	71°	5	48	5½ hrs.	
6	1 „	A.	1. vi. 18	71°	5	0	0	
7	1 „	B.	4. vi. 18	69°	3	20	0	
	·75 gr.	A.	8. vii. 18	73°	23	69	5 hrs.	
8	1 gr.	A.	5. vi. 18	69°	2	1	0	
9	1 „	B.	7. vi. 18	69°	0	13	0	
	·75 gr.	B.	28. vi. 18	70°	1	31	3 hrs.	
10	1 gr.	A.	10. vi. 18	67° 68°	0	7	3 „	
11	1 „	B.	11. vi. 18	68° 69°	0	20	4 „	
12	1 „	A.	14. vi. 18	69°	10	23	2 „	
13	1 „	B.	14. vi. 18	69°	16	30	1 „	G.T.
14		B.	17. vi. 18	66°	10	13	2½ „	G.T.
15	1 „	A.	17. vi. 18	66° 64°	0	11	19 „	G.T.
15 *a*	1 „	A.	30. vii. 18	75°	0	119	1 „	A.B.
17	·75 gr.	A.	25. vi. 18	66°	30	45	2 „	
18	2 c.c.	A.	28. vi. 18	68°	33	20	4 „	
19	3·5 grs.	B.	26. vii. 18	70°	52	30	5 „	A.B.
20	1 gr.	A.	6. viii. 18	69° 70°	22	19	2–4 hrs.	G.T.
21	1 „	A.	9. viii. 18	69° 70°	0	100	2½ hrs.	G.T.
22	1 „	A.	12. viii. 18	74°	22	91	2 „	G.T.
22 *a*	1 „	A.	13. ix. 18	64° 66°	0	21	2 „	G.T.

(1) Nos. 6 and 8 were not easy to apply, and the essential ingredients were better combined in No. 9.

(2) No. 19, owing to its volatile nature, precluded any possibility of using a measured quantity. The fluid was sprayed over the forearm until the skin was completely covered; about 3·5 grs. were needed, but there was some unavoidable waste.

(3) No. 22 was impossible to apply satisfactorily, even by the use of heat or water; most of it flaked off, but left a strong odour behind. Put up in altered form as 22 *a* it still left much to be desired as regards its spreading properties, after the lapse of an hour or more becoming too dry to adhere properly to the skin.

The preliminary trials indicated that some of the preparations showed the maximum degree of efficiency in relation to the conditions of the test, whereas others only afforded a partial degree of protection.

Between the first census of bites and a second one carried out on June 20th, when 39 bit in cage A, and 59 in cage B, there was apparently a considerable reduction in the biting population of the cages. It is not easy to estimate the amount of this reduction, or exactly when it occurred, owing to the early method of allowing no interval between the insertion of the treated and the control arm—which confused the issue. The reduction must however be attributed to the heavy death rate occasioned by drowning and drought. Subsequently, after the cages had been covered in by waterproof paper, and leaves had been placed in the water receptacles, the death rate was minimised and the population increased, any fluctuations being due either to the introduction of recently reared adults or to deaths owing to the natural completion of the life cycle.

The early tests were repeated after the population had increased, so that the trials of the preparations were brought into conformity with the others. In Table I both tests will be found bracketed against the preparation.

In a few tests (Preparations Nos. 10, 11, 15, 20, 21 and 22) the temperature altered during the period between test and control; in such cases both temperatures are recorded.

IX. EXPERIMENTS, SERIES II.

The Duration of the Period of Effectiveness.

For the purpose of military use it is of special importance that the protection afforded should cover as long a period as possible. A further series of trials was therefore arranged in order to determine for what period protection would be afforded.

Preliminary trials in connection with this series showed that it was useless to expect protection to be conferred for a longer period than a few hours at most, the better preparations affording practically no protection after the lapse of 16 and 8 hours respectively. The period of trial was therefore reduced first to 5, then to 3, and finally to 2 hours.

The efficient culicifuges, comprising Nos. 1, 2 *a*, 5, 9 *a*, 9 *b*, 10, 11, 15 *a*, 21 and 22 *a* were alone included in this series of trials. The results obtained are given in Table II.

Table II.

The Duration of the Period of Efficiency of certain Culicifuges.

Preparation used	Quantity used	Cage	Date	Room temperature F.	2 hrs after application: No. of bites on treated arm	2 hrs after application: No. of bites on untreated arm	3 hrs after application: No. of bites on treated arm	3 hrs after application: No. of bites on untreated arm	5 hrs after application: No. of bites on treated arm	5 hrs after application: No. of bites on untreated arm	Tested by
1	·75 gr.	B.	19. vii. 18	71°	12	30	—	—	—	—	A.B.
1	·75 ,,	A.	15. vii. 18	70°	—	—	—	—	41	17	G.T.
2 *a*	1 ,,	B.	2. viii. 18	69°	8	76	—	—	—	—	A.B
2 *a*	1 ,,	B.	6. viii. 18	69° 70°	27	42	—	—	—	—	G.T.
5	·75 ,,	A.	23. vii. 18	72°	36	37	—	—	—	—	G.T.
9 *a*	1 ,,	B.	13. viii. 18	75°	29	48*	—	—	—	—	G.T.
9 *a*	·75 ,,	B.	12. vii. 18	70°	—	—	—	—	50	not controlled	A.B.
9 *b*	1 ,,	B.	23. vii. 18	72°	—	—	35	46	—	—	A.B.
10	·75 ,,	B.	16. vii. 18	71°	49	68	—	—	—	—	A.B.
11	·75 ,,	A.	19. vii. 18	71°	81	not controlled	—	—	—	—	G.T.
15 *a*	1 ,,	A.	2. viii. 18	69°	37	74	—	—	—	—	G.T.
15 *a*	1 ,,	B.	30. vii. 18	75°	—	—	21	64†	—	—	A.B.
21	1 ,,	B.	10. viii. 18	75°	27	68	—	—	—	—	A.B.
22 *a*	1 ,,	A.	16. ix. 18	67°	25	19	—	—	—	—	G.T.

* Control made 10 minutes after test. † Control made within a few minutes of test.

Of the preparations used in the tests shown in Table II, with the exception of the proprietary ones, Nos. 10 and 11 (which were unaltered), those not already made up with wax were recompounded in conformity with the others. This change was necessary in order to prevent their being at a disadvantage in the long period trials in comparison with Nos. 1, 5, 10 and 11.

X. COMPARATIVE EFFICIENCY OF THE PREPARATIONS TESTED.

A percentage of the bites obtained in the trials set forth in Table II, out of the total number recorded gives an index to the comparative efficiency of these preparations. This index is shown in Table III.

Table III.

Index of Comparative Efficiency of Culicifuges.

Preparation tested	Time after application: 2 hours	3 hours	5 hours	Percentage of bites	Tested by
1	×	—	—	28	A.B.
1	—	—	×	70	G.T.
2 *a*	×	—	—	9	A.B.
2 *a*	×	—	—	39	G.T.
5	×	—	—	49	G.T.
9 *a*	×	—	—	37	G.T.
9 *b*	—	×	—	43	A.B.
10	×	—	—	43	A.B.
15 *a*	—	×	—	24	A.B.
15 *a*	×	—	—	33	G.T.
21	×	—	—	28	A.B.
22 *a*	×	—	—	56	G.T.

No importance is to be attached to the difference in the absolute number of bites as between one preparation and another, owing to the fluctuation in the number of hungry female mosquitoes present. We would also point out that the individual idiosyncrasies of the two experimenters in respect of their attraction for *Stegomyia fasciata*, may have influenced the results. It will be noted in the two parallel tests with No. 2 *a*, that the percentage of bites is very different; in the case of A. B. only 9 % bit, while in the case of G. T. 39 % bit. The only other test that permits of comparison in this respect is in the case of No. 15 *a*; in the two hours test 33 % bit G. T., while in a *three* hours test only 23 % bit A. B.

If we analyse the first case, that of 2 *a*, we notice that, whereas A. B. tested it on August 2nd, G. T. tested four days later on August 6th, the same cage being used. On August 1st about 16 females were added to the cage and these would normally bite on the 3rd and certainly on the 6th. On August 2nd, about 12 females were added, which would also bite on the 6th. We have therefore an addition of about 28 females ready to bite on the 6th. It happened, however, that a total of only 69 bit on the second occasion, as against 84 on the first. Allowing for the greater attraction, subsequently shown to be possessed by G. T. for the mosquito (see Section XII), the difference in the total number of bites in the two results must be mainly due to some mortality between the two dates, or to some unexplained disinclination to suck blood on the part of a number of the females.

If we omit the question of the difference of attractiveness of A. B. and G. T., the value of the preparations tested would rank, on the basis of Table III, as follows: 2 *a*, 1, 15 *a*, and 21 almost on a parity, followed by 9 *a*, 10 and 5 in order, but if an allowance be made for individual attractiveness, the figures point to 15 *a* as the most efficient culicifuge.

The following classification of the preparations is in accordance with the percentage of bites in relation to the total number received in test and control. In drawing it up no allowance has been made for the greater preference of the insects for one of the experimenters over the other, as it was only in the later series of trials that this preference was noted and recorded. As shown in the discussion of the results of the long period trials this factor may be of considerable importance and in judging the results allowance must be made for it.

1. Preliminary Trials.

0 %. Nos. 1, 2, 10, 11, 15, 15 *a*, 21 and 22 *a*.
3 %. No. 9 (in the first trial no bites were recorded).
9 %. No. 5 (on result of second trial).
10 %. No. 3 (on result of second trial).
19 %. No. 22.
25 %. No. 7 (on result of second trial).
26 %. No. 4 (on result of second trial).
30 %. No. 12.
35 %. No. 13.

40 %. No. 17.
43 %. No. 14.
53 %. No. 20.
62 %. No. 18.
63 %. No. 19.

2. Long Period Trials.

2 *hours.*

28 %. Nos. 1 and 21.
29 %. No. 2 *a* (combined result of two trials: A. B. shows 9 %. G. T. shows 39 %).
33 %. No. 15 *a*.
38 %. No. 9 *a*.
42 %. No. 10.
49 %. No. 5.
56 %. No. 22 *a*.

3 *hours.*

25 %. No. 15 *a*.
43 %. No. 9 *b*.

5 *hours.*

71 %. No. 1.

XI. BEHAVIOUR OF THE MOSQUITO IN THE PRESENCE OF THE CULICIFUGE.

In the cage the untreated arm is generally attacked by the insects after the lapse of not longer than two minutes. A treated arm, where the preparation used is not effective, is attacked within the same period. After a lapse of five minutes all the hungry females will have generally attacked.

We noted, during the first series of tests (see Table I) that with effective preparations, the insects sometimes settled on the treated arm and walked over it, flying off again without feeding, being apparently indifferent to the odour.

Again, with most of the preparations the wounds occurred on the wrist and towards the elbow, such localised attacks being possibly due to the proximity of the untreated areas of hand and arm.

On one occasion, in applying a preparation, a narrow streak was inadvertently left untreated, and the insects not only bit on that area, *but also through the preparation in the area immediately adjacent*, no bites being found elsewhere.

Our observations therefore show that the preparations are only likely to be effective within a very restricted range. Moreover, the entire exposed area must be covered, and steps must be taken for renewed application, even with the best of the culicifuges experimented with, at intervals of not more than two hours.

On the basis of these observations we are inclined to put forward a tentative theory to the effect that the action of the culicifuges is rather to obscure the human odour than to repel the insects.

XII. EXPERIMENTS CONCERNING THE DEGREE OF ATTRACTION EXHIBITED BY DIFFERENT INDIVIDUALS FOR MOSQUITOES.

It is a matter of general comment that some individuals are more prone to mosquito attacks than others. Finlay (1912) records that natives of Africa are least liable to attack and that people of northern races recently arrived in the tropics are most persecuted; he considered it probable that this preference depended upon the thickness of the skin, and the condition of the capillary circulation of the skin. In regard to the Yellow Fever mosquito (the species used in these experiments) Goeldi (1912) considered that the presence of perspiration attracted both sexes. The French investigators, Marchoux, Salimbeni and Simond (1912), concluded from experiments that, although *Stegomyia fasciata* readily bites both negro and red skinned races, it shows a preference for white skinned people. They further showed that this mosquito exhibited preferences among whites for young and vigorous persons, over aged, and anaemic ones. It is also very generally asserted that in the tropics seasoned whites enjoy a certain amount of immunity at the expense of fresh arrivals.

As this factor of preference on the part of the mosquitoes for one of the experimenters was apparently influencing our results (see pp. 234–5), we planned a series of experiments in order to ascertain if possible some measure of its importance, and carried them out as opportunity permitted. The method adopted was similar to that already described in regard to the testing of culicifuges (see Section VII), but for the purpose of these trials two persons exposed their untreated arms simultaneously in the same cage for 10 minutes.

The following data relating to the persons who exposed their arms in the cages may be of interest.

A. B. *Age* 51. Has never resided out of England until three years ago when he spent 13 months in W. Africa. During and since that period has regularly allowed mosquitoes to feed upon his arms. Shows very slight reaction to bites.

G. T. *Age* 35. Save for two months' residence in N. France, has always lived in England. Has allowed mosquitoes to bite him frequently during the past four months, and showed a marked reaction to their bites, but seems to have gained some measure of immunity since the commencement of this research.

H. *Age* 38. Born in Australia and lived for many years in Queensland; has resided in England for the past six years. Shows very slight reaction to bites.

I. *Age* 48. Fourteen years ago resident off and on in Tropical South America for about ten years. Has since been resident in England. Shows marked reaction to bites.

J. *Age* 18. Has never resided out of England. Shows a marked reaction to bites.

K. *Age* 27. Has never resided out of England. Shows a marked reaction to bites.

L. *Age* 54. About a year ago resided for 13 months either in Greece or Malta. Has never lived in the tropics. Shows slight reaction to bites.

M. *Age* 31. Never resident out of England. Shows a marked reaction to bites.

Table IV.

Number of experiment	Initial of experimenter	Number of bites	Percentage of total bites	Initial of experimenter	Number of bites	Percentage of total bites	Total No. of bites recorded
1	A.B.	10	17 %	G.T.	49	83 %	59
2	A.B.	3	8	G.T.	36	92	39
3	A.B.	37	84	H.	7	16	44
4	A.B.	16	38	I.	27	62	43
5	A.B.	23	47	J.	26	53	49
6	A.B.	16	25	K.	50	75	66
7	A.B.	6	30	L.	14	70	20
8	G.T.	6	14	K.	38	86	44
9	G.T.	8	30	J.	19	70	27
10	G.T.	62	78	H.	17	22	79
11	G.T.	27	77	I.	8	23	35
12	G.T.	4	25	M.	12	75	16

Out of the six persons who assisted in these trials two, owing to lack of opportunity, made the trial with one of the experimenters only, L. with A. B. and M. with G. T. and their records are consequently discarded when making a final comparison between the resistant powers of A. B. and G. T. For this purpose the percentages of the results may be arranged as under. The two trials between A. B. and G. T. are averaged and treated as a single trial.

Table V.

	A.B.	G.T.	H.	I.	J.	K.	Totals
A.B.	—	12·5	84	38	47	25	206·5
G.T.	87·5	—	78	77	30	14	286·5
H.	16	22					
I.	62	23					
J.	53	70					
K.	75	86					
	206	201					

Totals of the percentage of bites received by the four experimenters H., I., J. and K. in trials with A.B. and G.T. respectively.

From this it would appear that in the comparison of A. B. and G. T. alone the ratio of the mosquitoes' preference for G. T. is roughly 7 to 1, but if the question is judged in relation to the other four experimenters in combination with A. B. and G. T. the ratio of preference is less than 3 to 2; while, if the comparison is made through the relation of the four persons H., I., J. and K. only, leaving the direct tests between A. B. and G. T. out of account, the preference is not far from parity. It is probable that this divergence in result, according to the inclusion or exclusion of some of the experimenters, is not a matter of pure chance, but the effect of the mosquitoes' preference being swayed by more than one factor; for instance, if the presence of a moist skin is one factor the temperature of the skin may constitute another, and the intensity or other characteristic of the odour arising from the skin yet another factor.

As regards the moisture and temperature of the skin, some allowance is no doubt necessary for the individual periodic fluctuation of A. B. and G. T., but it does not seem probable that this could be of sufficient magnitude, especially under laboratory conditions, to greatly modify the results.

Our conclusion is therefore that some allowance must be made for the greater attraction of G. T. over A. B., and that for practical purposes it is safest to assume that this ratio is more likely to be 3 to 2 than either of the extremes.

XIII. SUMMARY OF RESULTS.

For the purpose of testing culicifuges numbers of *Stegomyia fasciata* (the Yellow Fever mosquito), bred in incubators, were placed in cages kept in the laboratory. The efficiency of the preparations was tested by coating the forearm with a definite quantity of one or other of them and exposing in a cage, each test being controlled by inserting after a short interval the other and untreated arm in the same cage, the number of bites being compared in each case. By this procedure it was hoped to obtain a measure of the relative protection which the culicifuges might be expected to afford when used under practical conditions against *Anopheles* mosquitoes.

In the first series of trials conducted within 15 minutes of application, eight preparations out of a total of 22 tested gave satisfactory results. Their active ingredients were (1) Oil of Cassia and Camphor, (2 *a*) Oil of Cassia and Peppermint, (5) Oil of Eucalyptus and Citronella with Phenol, (9 *a*) Crude Naphthalene (coke oven) and Camphor, (10) Crude "Parasitox," (15 *a*) Light Wood Oil, (21) Oil of Turpentine, (22 *a*) "Lawson's Anti-mosquito Compound." These preparations were then tested to ascertain for what period their protection could be depended upon. Preliminary trials indicated that this period was not likely to extend for more than two hours between treatment of the arm and exposure in the cage. None of the preparations gave complete protection in this series of trials; the most efficient were Nos. 1, 21, 2 *a*, 15 *a* and 9 *a*, over a two hours period.

It appeared, however, that the two experimenters were unequally attractive to the mosquito. Making allowance for this, preparations 2 *a*, 1, 15 *a*, and 21 were almost on a parity, followed by 9 *a*, 10 and 5 in order. A series of experimental trials based on this element of preference on the part of the mosquito was confirmatory of the factor of preference and showed a ratio of attraction of about the order of G. T. 3, A. B. 2.

Observations on the behaviour of the mosquitoes during the tests suggest that the protection afforded does not result from a dislike of the insects to the culicifuge but to its obscuring the attractiveness of the human odour.

Regarding the make up of the essential ingredients it was found that fluid preparations were inconvenient and tended to be wasteful in application. Soap preparations, unless very soft, are apt to be difficult of economic use and in either case are more readily dissipated by perspiration than waxy or greasy

ones. Culicifuges prepared with grease are more easily applied but are not so lasting as those put up with wax. Soft wax preparations, correctly adjusted to the temperature in which it is proposed to use them, are most suitable for out of doors use, especially in the case of troops. The retarding of volatilisation caused by the admixture of the active ingredients with wax or grease is a distinct advantage, but care must be exercised in respect of the relative proportion of active substance to the base. The golden rule is to use as much of the active constituent and as small a quantity of the inert base as is consistent with convenient application and the prolonging of the period of efficiency, not, as most proprietary compounders appear to think, as little as possible.

In choosing a culicifuge for use, the human factor of choice in regard to pleasing and unpleasing odours should be as little disregarded as consistent with efficiency. Where large quantities are needed cost is an important consideration, but must not be allowed to endanger the willing co-operation of the individuals it is desired to protect.

REFERENCE.

HOWARD, DYAR and KNAB (1912). *The Mosquitoes of North and Central America and the West Indies*, vol. I. pp. 260, 261. Carnegie Institute. Washington.

SUPPLEMENTARY NOTE ON THE FORMATION OF A COCOON BY CYCLORHAPHOUS DIPTEROUS LARVAE.

By D. KEILIN, Sc.D.

(*From the Quick Laboratory, University of Cambridge.*)

The formation of a cocoon or of a more or less solid case by a larva before its transformation into a pupa, occurs very seldom in cyclorhaphous Diptera. This is due undoubtedly to the existence in all these Diptera of a puparium formed by the contracted and hardened last larval skin, in which, as in a cocoon, is contained the real pupa or chrysalis of the fly.

In the appendix to my paper on Anthomyid larvae[1] I gave a complete account of all that is known of cocoons in cyclorhaphous Diptera. This pertained to but five species: *Myospila meditabunda* F., *Mydaea pertusa* Meig., *M. anomala* Jaen., *M. pici* Macq. and *Passeromyia heterochaeta* Villeneuve.

In the same paper, when dealing with the life-history of *Muscina stabulans* Fall., I referred to Dufour's paper (1840)[2] in which he stated that the pupa of this fly, bred from *Agaricus campestris*, were surrounded by a cocoon formed by a paste of moistened paper which was left with the fungi in one of his breeding-jars.

As this observation was not confirmed by other entomologists who studied *M. stabularis*, I considered it as somewhat doubtful. I am now able, however, to assert that Dufour's observation was correct and that it was recently confirmed independently by Dr G. S. Graham-Smith and Mr James E. Collin. I owe the following information relating to *Muscina pabulorum* Fall. and *M. stabulans* Fall., during the year 1916, to the courtesy of Dr Graham-Smith.

1. "Females of *M. pabulorum* Fall. were placed in a glass jar, covered with wire cloth, containing the body of a recently killed guinea-pig.... The puparia were found in detached masses of hair arranged like the cells in a honeycomb, each puparium lying in a tough case mainly composed of hairs."

[1] *Parasitology*, IX. 325–450 (1917).

[2] Dufour (1840). "Second mémoire sur les métamorphoses de plusieurs larves fongivores appartenant à des Diptères." *Ann. de Sc. Nat.* 2e série, XIII. 148–163, Pl. III. On page 157 of this paper we read as follows:

"Au moment de leur transformation en pupe, ces larves m'ont fourni un fait assez singulier. Dans le bocal où je les élevais, j'avais laissé du papier qui, incessamment humecté par la décomposition du champignon, se reduisit en pâte. Je trouvais plusieurs pupes enveloppées d'une espèce de cocon ou de fourreau formé avec cette pâte; mais toujours le bout antérieur, celui par lequel dévait avoir lieu l'éclosion, était découvert. D'autres pupes étaient nues au milieu du magma ou dans le sable qui garnissait le fond du vase."

2. "Some large larvae of *M. stabulans* Fall. were placed in a jar containing sand. On examination some days later, small oval lumps were seen amongst the loose sand. These lumps consisted of grains of sand cemented together to form cases round the puparia."

Similar cases, containing the puparia of *M. stabulans*, were also recently found by Mr Collin, who kindly sent me a couple of these cases which enabled me to confirm the previous observations. The cases received from Mr Collin are exceedingly fragile and composed of small grains of sand cemented together with an imperceptible secretion; the cases are oval in form with a rough external surface and smooth inside.

As the formation of a cocoon or a case is now proved for two species of the genus *Muscina*, *M. stabulans* and *pabulorum*, it is surprising that it has not yet been found in another very closely related species, *M. assimilis* Fall., of which I have examined a number of larvae and pupae, without noticing the slightest trace of a cocoon or a case. It is possible, however, that under other conditions than those which prevailed in my experiments the larvae of this species may produce a cocoon which surrounds the puparium.